VOLUME 38 JULY 1960 NUMBER 4

Canadian Journal of Botany

Editor: D. L. BAILEY

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

Canadian Journal of Botany

Under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research, the National Research Council issues THE CANADIAN JOURNAL OF BOTANY and five other journals devoted to the publication, in English or French, of the results of original scientific research. Matters of general policy concerning these journals are the responsibility of a joint Editorial Board consisting of: members representing the National Research Council of Canada; the Editors of the Journals; and members representing the Royal Society of Canada and four other scientific societies.

EDITORIAL BOARD

Representatives of the National Research Council

I. McT. COWAN (Chairman), *University of British Columbia*
H. G. THODE, *McMaster University*
D. L. THOMSON, *McGill University*
L. MARION, *National Research Council*

Editors of the Journals

D. L. BAILEY, *University of Toronto*
T. W. M. CAMERON, *Macdonald College*
H. E. DUCKWORTH, *McMaster University*
LÉO MARION, *National Research Council*
J. F. MORGAN, *Department of National Health and Welfare, Ottawa*
R. G. E. MURRAY, *University of Western Ontario*
J. A. F. STEVENSON, *University of Western Ontario*

Representatives of Societies

D. L. BAILEY, *University of Toronto*
Royal Society of Canada
T. W. M. CAMERON, *Macdonald College*
Royal Society of Canada
H. E. DUCKWORTH, *McMaster University*
Royal Society of Canada
Canadian Association of Physicists
P. R. GENDRON, *University of Ottawa*
Chemical Institute of Canada
J. F. MORGAN, *Department of National Health and Welfare, Ottawa*
Canadian Biochemical Society
R. G. E. MURRAY, *University of Western Ontario*
Canadian Society of Microbiologists
J. A. F. STEVENSON, *University of Western Ontario*
Canadian Physiological Society
D. J. LEROY, *University of Toronto*
Royal Society of Canada

Ex officio

LÉO MARION (Editor-in-Chief), *National Research Council*
J. B. MARSHALL, Division of Administration and Awards, *National Research Council*

Manuscripts for publication should be submitted to Dr. D. L. Bailey, Editor, Canadian Journal of Botany, Department of Botany, University of Toronto, Toronto, Ontario.

(For instructions on preparation of copy, see **NOTES TO CONTRIBUTORS** (inside back cover).)

Proof, correspondence concerning proof, and orders for reprints should be sent to the Manager, Editorial Office (Research Journals), Division of Administration and Awards, National Research Council, Ottawa 2, Canada.

Subscriptions, renewals, requests for single or back numbers, and all remittances should be sent to Division of Administration, National Research Council, Ottawa 2, Canada. Remittances should be made payable to the Receiver General of Canada, credit National Research Council.

The journals published, frequency of publication, and subscription prices are:

Canadian Journal of Biochemistry and Physiology	Monthly	$ 9.00 a year
Canadian Journal of Botany	Bimonthly	$ 6.00
Canadian Journal of Chemistry	Monthly	$12.00
Canadian Journal of Microbiology	Bimonthly	$ 6.00
Canadian Journal of Physics	Monthly	$ 9.00
Canadian Journal of Zoology	Bimonthly	$ 5.00

The price of regular single numbers of all journals is $2.00.

Canadian Journal of Botany

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 38 JULY 1960 NUMBER 4

FUNGI AND DECAY IN ASPEN AND BALSAM POPLAR IN THE BOREAL FOREST REGION, ALBERTA[1]

G. P. THOMAS,[2] D. E. ETHERIDGE,[3] AND GLEN PAUL[4]

Abstract

Examination of 835 living trembling aspen (*Populus tremuloides* Michx.) and 527 balsam poplar (*Populus balsamifera* L.) on 47 sample plots of mixed-wood forest of the Boreal Forest Region, Alberta, showed 73% of the aspen and 61% of the balsam poplar to be decayed. Additional trees of both species were infected by stain-producing fungi. The incidence of infection in aspen decreased from mesic sites to dry sites, while that for balsam poplar was about the same in mesic and wet sites. *Fomes igniarius* (L. ex Fr.) Kickx was the first ranking fungus in both hosts in terms of associated decay. It had the greatest incidence of all fungi in aspen but ranked third to *Pholiota spectabilis* (Fr.) Quél. and *Polyporus adustus* Willd. ex Fr. in balsam poplar. *Corticium polygonium* Pers. and *Radulum casearium* (Morg.) Lloyd together caused about as much decay in aspen as did *Fomes igniarius*, although neither fungus occurred in balsam poplar. Contrary to its generally low incidence in balsam poplar, *Pholiota destruens* (Brond.) Quél. caused most of the decay in this host, excepting that ascribed to *Fomes igniarius*. *Pholiota destruens* did not occur in aspen. Many fungi caused both basal and stem infections in both hosts, while few caused infections of one type only. *Armillaria mellea* (Vahl ex Fr.) Quél. caused the greatest number of basal infections in aspen but was exceeded by *Pholiota spectabilis* in balsam poplar. The greatest amount of butt decay was ascribed to *Phlebia strigosozonata* (Schw.) Lloyd in aspen and to *Pholiota spectabilis* in balsam poplar. The overall losses of volume due to decay were 25% and 10%, respectively, for aspen and balsam poplar.

Introduction

The occurrence of heart rot in excessive amounts in aspen and balsam poplar in Alberta is a factor limiting the utilization of these species. It is partly on this account that less than 1% of the annual cut of timber in Alberta is comprised of aspen and balsam poplar, although the aggregate amount of timber of these trees approximates 48% of the provincial inventory of all species.

[1]Manuscript received February 24, 1960.
Contribution No. 620 from the Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada.

[2]Forest Biology Laboratory, Calgary, Alberta.
[3]Forest Biology Laboratory, Quebec, P.Q.; formerly at Forest Biology Laboratory, Calgary, Alberta.
[4]Forest Surveys and Inventory Division, Department of Lands and Forests, Edmonton, Alberta.

[The previous number of Can. J. Botany (**38**, 267–457) was issued May 12, 1960.]

Both aspen and balsam poplar are widely distributed in the province, but each attains its best development in the mixedwood forests of the Boreal Forest Region, that is, in central and northern Alberta (6). Because of the preponderance of these two species in the region indicated, the Alberta Department of Lands and Forests, in co-operation with the Federal Forest Biology Division, in 1957 began a survey of commercial stands of poplar to obtain cull factors having general application to provincial and industrial requirements. The survey was completed in 1959, and among other information obtained was an appreciation of the identity and distribution of fungi that are associated with decay in living trees. A report on interim findings of the survey was issued in 1958 (4). This paper discusses the completed survey from the point of view of the fungi that enter into the decay complex of aspen and balsam poplar in selected areas.

Method of Study

Stands of aspen and balsam poplar were examined in eight localities within the general area of Slave Lake – Whitecourt – Grande Prairie, taking into account a range of forest sites and tree ages. Three sites were recognized on the basis of their physiography, topography, soil, and vegetation, as follows: *dry*, *mesic*, and *wet*. Aspen occurred as pure stands in dry sites and either as pure stands or in mixture with balsam poplar in mesic sites. Balsam poplar occurred as pure stands in wet sites and either as pure stands or in mixture with aspen in mesic sites. Spruce (*Picea glauca* (Moench) Voss) and pine (mainly *Pinus contorta* Dougl. var. *latifolia* Engelm.) were minor stand components in all areas. Spruce occurred mainly in mesic and wet sites and pine in dry sites.

A total of 835 living aspen on 28 sample plots in seven areas and 527 living balsam poplar on 32 sample plots in six areas were examined for the presence of rot by dissectional means. All of the trees were larger than 3.5 in. in diameter 4.5 ft above the ground. The ages of aspen varied from 23 to 117 years (av. 61 years) and those for balsam poplar from 24 to 158 years (av. 65 years). The locations and patterns of decayed wood as indicated at log ends were noted for each tree. Trees were sectioned at frequent and irregular intervals for this purpose and the resulting measurements of individual rots were converted to cu.-ft volumes using Smalian's formula.

Where possible each rot was tentatively identified by associating it with fruit bodies. Final identifications were made by cultural means,[5] samples of decayed wood being collected for this purpose. When two or more fungi were isolated from a sample of decayed wood the rot represented by that sample was termed a *mixed rot*. When only non-basidiomycetous fungi were isolated from samples of decayed wood the rots in question were termed *unknown rots*.

[5]The authors are indebted to Dr. Mildred K. Nobles, Plant Research Institute, Research Branch, Department of Agriculture, Ottawa, and her associates, for identifying many of the fungi encountered in the investigation.

Results

Incidence of Wood-rotting Fungi in Aspen and Balsam Poplar

A higher level of infection of known wood-rotting fungi occurred in aspen than in balsam poplar (Table I). The difference between the two species resulted mainly from the high incidence of infection in aspen in mesic sites, the levels of infection in dry-site aspen, mesic-site balsam poplar, and wet-site balsam poplar being fairly comparable. The reasons underlying the observed differences in infection levels between sites and species of host were not readily apparent and are the subject of continuing investigation.

TABLE I

The incidence of wood-rotting fungi in aspen and balsam poplar in Boreal Forests, Alberta

Site	Number of plots	Total sample			Infected trees			Percentage of sample infected
		Number	Age (years) Range	Av.	Number	Age (years) Range	Av.	
			Aspen					
Dry	14	606	23–117	61	420	25–117	70	69.3
Mesic	14	229	23–112	61	189	23–112	67	82.5
Total	28	835	23–117	61	609	23–117	69	72.9
			Balsam poplar					
Mesic	14	222	24–105	62	139	25–104	70	62.6
Wet	18	305	24–158	69	184	26–158	87	60.5
Total	32	527	24–158	65	323	25–158	79	61.0

A further demonstration of the relative conditions of the two species was gained by comparing the infection levels for each in a number of stands of similar ages on comparable (mesic) sites. These comparisons showed the incidence of infection in aspen to be consistently greater than that for balsam poplar, although the differences between the two were progessively less in older stands. Thus, aspen was about 78% infected and balsam poplar about 30% infected in 50-year-old stands in mesic sites. The levels of infection in 80-year-old stands were 85% for aspen and 75% for balsam poplar.

Although aspen would appear to be more prone to infection by decay fungi than is balsam poplar, the reverse was true for non-basidiomycetous fungi that were associated with heartwood stains. The higher incidence of heartwood stains in balsam poplar than in aspen resulted largely from the occurrence in balsam poplar of an "imperfect fungus", identified as *Libertella* sp. This fungus was relatively uncommon in aspen. The stain associated with *Libertella* sp. was reddish brown in color in most instances and was usually located close to branches or branch stubs. The same fungus was reported by Basham (1) to be associated with a red, mottled stain of aspen in Ontario, and by Fritz (3) with a rot of characteristic pattern in stored aspen and poplar pulpwood in Ontario. Fritz demonstrated by laboratory experiments that the fungus, designated as "fungus X", was capable of attacking sound, autoclaved

poplar wood. Basham, however, was unable to find that *Libertella* sp. can cause decay. Limited tests of the fungus on aspen at Calgary, using wood sterilized by propylene oxide gas, showed no weight loss to occur after 3 months. Apparently, therefore, the decay status of *Libertella* sp. is uncertain. Accordingly, it was assumed that *Libertella* sp. does not cause decay normally associated with known wood rotters. It is largely on this account that balsam poplar was considered to be less heavily infected by wood-rotting fungi than was aspen.

Fungi Associated with Decay in Living Aspen and Balsam Poplar

The occurrence and relative importance of the fungi encountered in the survey were as shown in Tables II and III, which also show the associations of individual fungi with rots originating in either the butts or trunks of trees.

TABLE II

Fungi associated with decay of living aspen in Boreal Forests, Alberta

Organisms	Infections		Rot	
	Number	Percentage of total	Volume (cu. ft)	Percentage of total
Butt infections	239	31.5	248.7	10.8
Armillaria mellea (Vahl ex Fr.) Quél.	55	7.3	18.5	0.9
Collybia velutipes (Curt. ex Fr.) Quél.	1	0.1	Tr.	Tr.
Corticium polygonium Pers.	42	5.6	33.4	1.5
Fomes fomentarius (L. ex Fr.) Kickx	3	0.4	0.1	Tr.
**F. igniarius* (L. ex Fr.) Kickx	2	0.3	5.9	0.2
F. pinicola (Sw. ex Fr.) Cke.	1	0.1	0.1	Tr.
Hymenochaete tabacina (Sow. ex Fr.) Lév.	3	0.4	0.9	Tr.
Phlebia strigosozonata (Schw.) Lloyd	13	1.7	60.3	2.2
Pholiota adiposa Fr.	18	2.4	4.9	0.2
P. spectabilis (Fr.) Quél.	20	2.6	27.3	1.2
P. subsquarrosa (Fr.) Quél.	2	0.3	8.1	0.4
Polyporus adustus Willd. ex Fr.	6	0.7	15.6	0.7
P. zonatus Fr.	1	0.1	0.1	Tr.
Radulum casearium (Morg.) Lloyd	7	0.9	27.5	1.2
Trechispora raduloides (Karst.) Rog.	2	0.3	0.1	Tr.
Mixed	12	1.6	15.2	0.7
Unknown	51	6.7	30.7	1.4
Trunk infections	520	68.5	2053.5	89.2
Corticium laeve Pers. ex Fr.	1	0.1	0.2	Tr.
C. polygonium Pers.	101	13.3	293.5	12.8
Fomes fomentarius (L. ex Fr.) Kickx	1	0.1	0.4	Tr.
**F. igniarius* (L. ex Fr.) Kickx	152	20.1	793.0	34.4
Hymenochaete tabacina (Sow. ex Fr.) Lév.	1	0.1	Tr.	Tr.
Merulius tremellosus (Schraed. Fr.	1	0.1	Tr.	Tr.
Pholiota adiposa Fr.	1	0.1	11.2	0.5
P. spectabilis (Fr.) Quél.	2	0.2	3.0	0.1
Polyporus adustus Willd. ex Fr.	16	2.1	34.4	1.5
P. zonatus Fr.	9	1.3	47.8	2.1
Radulum casearium (Morg.) Lloyd	56	7.4	326.1	14.2
Trechispora raduloides (Karst.) Rog.	4	0.5	12.2	0.5
Mixed	112	14.8	285.0	12.4
Unknown	63	8.3	246.7	10.7
Total	759	100.0	2302.2	100.0

*Includes *Fomes igniarius* (L. ex Fr.) Kickx and *F. igniarius* (L. ex Fr.) Kickx var. *populinus* (Neuman) Campbell.

TABLE III

Fungi associated with decay of living balsam poplar in Boreal Forests, Alberta

Organism	Infections		Rot	
	Number	Percentage of total	Volume (cu. ft)	Percentage of total
Butt infections	167	37.5	248.7	10.5
Armillaria mellea (Vahl ex Fr.) Quél.	25	5.6	24.7	1.6
**Corticium expallens* Bres.	8	1.8	6.1	0.4
C. vellereum Ellis & Cragin	7	1.6	1.4	0.1
Pholiota destruens (Brond.) Quél.	2	0.4	6.8	0.4
P. spectabilis (Fr.) Quél.	41	9.2	64.0	4.2
Polyporus adustus Willd. ex Fr.	11	2.5	2.1	0.1
Stereum purpureum (Pers. ex Fr.) Fr.	4	0.9	1.1	0.1
Trechispora raduloides (Karst.) Rog.	5	1.1	0.9	0.1
Unknown	64	14.4	52.7	3.5
Trunk infections	278	62.5	1359.3	89.5
Armillaria mellea (Vahl ex Fr.) Quél.	1	0.2	Tr.	Tr.
Coprinus micaceus Fr.	1	0.2	Tr.	Tr.
**Corticium expallens* Bres.	5	1.1	79.0	5.2
C. vellereum Ellis & Cragin	3	0.7	4.7	0.3
†*Fomes igniarius* (L. ex Fr.) Kickx	43	9.7	407.2	26.8
Pholiota destruens (Brond.) Quél.	24	5.4	269.5	17.7
P. spectabilis (Fr.) Quél.	13	2.9	18.5	1.3
Polyporus adustus Willd. ex Fr.	35	7.9	37.3	2.5
Trametes trogii Berk.	1	0.2	2.4	0.2
Trechispora raduloides (Karst.) Rog.	7	1.6	12.8	0.8
Unknown	145	32.6	527.5	34.7
Total	445	100.0	1519.1	100.0

*Tentative identification only.
†Includes *Fomes igniarius* (L. ex Fr.) Kickx and *F. igniarius* (L. ex Fr.) Kickx var. *populinus* (Neuman) Campbell.

A large number of infections that had resulted in rot could not be connected with fungi of known decaying ability. Thus, 47% of the infections noted for balsam poplar remained unidentified, largely because the mycoflora of many rots was dominated by hyphomycetous fungi. The incidence of unidentifiable rots in aspen was less than that for balsam poplar—about 15%. However, an additional 16% of the infections in aspen had resulted in a condition of mixed rots, mainly of *Corticium polygonium* and *Radulum casearium*. Although incipient rots of these species were distinctive from those of other fungi, their separate identification was uncertain unless cultures could be obtained.

The wood-rotting mycoflora observed for aspen differed from that for balsam poplar, as to both the kind and the number of different species involved (Table II and Table III). Of 17 species of wood-rotting fungi encountered in aspen and 11 in balsam poplar only five were common to both hosts, as follows: *Armillaria mellea*, *Fomes igniarius*,[6] *Pholiota spectabilis*, *Polyporus adustus*, and *Trechispora raduloides*. While basal infections accounted for about one third of all infections noted for both species of host, the average volume of rot per basal infection was low. Thus, the aggregate amount of rot

[6]Difficulties were experienced in providing separate identifications for *Fomes igniarius* (L. ex Fr.) Kickx and *F. igniarius* (L. ex Fr.) Kickx var. *populinus* (Neuman) Campbell because of apparent minor differences between these organisms in culture. Accordingly, both organisms were assigned to the former for purposes of this investigation.

associated with basal infections accounted for less than 11% of the total volume of rot in both hosts. The relatively small volume of butt rot encountered in aspen was associated, however, with more species of wood-rotting fungi than was the larger volume of trunk rot. Basal rots in balsam poplar on the other hand were associated with fewer fungi than were trunk rots.

Fomes igniarius was the first ranking fungus in terms of its associated rot in both hosts and was the species most frequently isolated from decayed aspen wood. The incidence of this fungus in balsam poplar was exceeded by *Pholiota spectabilis* and *Polyporus adustus*. Since about one third of the rot in aspen and more than one quarter of that in balsam poplar was attributed to *Fomes igniarius* alone, this fungus is important above all others in the successful management of both species in Alberta. Its significance in the management of aspen and poplar elsewhere in Canada has been clearly demonstrated (1, 2, 5).

Corticium polygonium and *Radulum casearium* caused about equal amounts of rot in aspen and were together responsible for more than one third of the rot encountered in this host. The incidence of *Corticium polygonium*, however, was more than double that of *Radulum casearium*. Neither fungus was encountered in balsam poplar. A comparison of the ages of trees infected by these two fungi showed that *Corticium polygonium* becomes established earlier than *Radulum casearium*, but that the rate of decay of the latter exceeds that of *Corticium polygonium*. Hence, at about 70 years of age the amount of rot ascribed to both species was about equal. The rots of both species are yellow and stringy in advanced stages and are difficult to separate. They are, however, readily distinguishable from comparable stages of *Fomes igniarius* rot which are white and spongy. The incipient stages of rot caused by *Corticium polygonium* and *Radulum casearium* could not be separated except by cultural means. The sporophores of *Corticium polygonium* were relatively common on branch stubs of living trees but were difficult to detect on standing trees because of their resupinate form and small size. Sporophores of *Radulum casearium* did not occur on living trees but were relatively common on dead trees and debris.

In contrast with its relatively low incidence *Pholiota destruens* was second only to *Fomes igniarius* as the cause of heart rot of balsam poplar. It was not encountered in aspen. Both of these fungi were associated mainly with a trunk rot. The rot associated with the former is yellow to tan, although sometimes darker in color, and is typically laminate. It is difficult to recognize other than in typical stages, incipient forms being manifested first by dark streaks in otherwise normal heartwood and later by small and irregularly shaped white patches. *Pholiota destruens* was identified with a major butt rot of black cottonwood (*Populus trichocarpa* Torr. & Gray) in British Columbia (7) but is otherwise unreported on Canadian species of *Populus*.

From the viewpoint of its frequency of isolation from butt rots *Armillaria mellea* ranked first in aspen and was second only to *Pholiota spectabilis* in balsam poplar. Many of the infections traced to *Armillaria mellea* had

originated in the roots of trees and resulted in very small volumes of rot. Hence, the aggregate amount of rot ascribed to this fungus was rather insignificant in both aspen and balsam poplar. Similarly, the amount of rot per infection of *Pholiota spectabilis* was usually small, although the incidence of this fungus exceeded that for all others in balsam poplar. It had a low incidence in aspen and was mainly associated with a butt rot in both host species. The butt-rotting fungus *Pholiota adiposa* on the other hand was found only in aspen.

The incidence of *Polyporus adustus* in balsam poplar was second only to that for *Pholiota spectabilis*, but was much lower in aspen. Most of the infections ascribed to this fungus occurred in the basal portions of trees, usually in association with extensive wounds as exemplified by scars. The average volume of rot per infection of *Polyporus adustus* was small, but because of the many infections ascribed to this fungus the aggregate amount of its associated rot in both species of host was considerable.

A fungus tentatively identified as *Corticium expallens* was associated with about 6% of the rot encountered in balsam poplar. The same fungus was found fruiting on slash and debris of black cottonwood in British Columbia, but was not isolated from decayed wood of this species (7). It was not found in aspen in the current study. The few infections of this fungus were about equally distributed between the butt and trunk regions. In contrast with its apparent low incidence the amount of rot per infection of *C. expallens* was generally large. Incipient stages of the rot were indistinguishable from normal heartwood. The typical form of the rot was uniformly white or yellowish in color and had a spongy texture. It closely resembled the rot of *Fomes igniarius* but lacked zone lines. The fruit bodies were resupinate, were yellow to tan in color, and grew on fallen branches and other debris. They were not observed on living trees.

The loss of volume due to decay caused by all species of fungi in aspen was much greater than in balsam poplar. Thus, aspen was about 25% decayed and balsam poplar about 10% decayed (cu.-ft basis). Most of the fungi referred to in this report are believed to confine their activities to living trees, or at least to be relatively inactive in dead trees and wood products. Known exceptions are *Polyporus adustus*, *Stereum purpureum*, and *Phlebia strigosozonata*, each of which may cause decay of stored aspen pulpwood (3).

Aspen was slightly more decayed in mesic sites than in dry sites, mainly because of the increased activity of *Fomes igniarius* in the former. Balsam poplar was slightly more decayed in wet sites than in mesic sites, mainly because of rot associated with *F. igniarius* and *Pholiota destruens*. Rot that could be ascribed to the latter fungus was almost negligible in mesic sites.

Acknowledgments

The authors express their appreciation to Mr. R. D. Loomis, formerly Senior Superintendent, Forest Surveys Branch, Department of Lands and Forests, Edmonton, for his active support of the project upon which this

report is based. They are also indebted for the assistance of the Superintendents of Forest Divisions of the Alberta Forest Service and to industrial foresters in whose administrative territories the work was carried out. The authors are grateful to Dr. Mildred K. Nobles, Senior Mycologist, Plant Research Institute, Department of Agriculture, Ottawa, and her associates, who identified many of the isolated fungi. They are indebted also to Miss R. C. Robinson and Mrs. E. J. McLeod of the Calgary Laboratory for isolating and identifying many of the fungi.

References

1. BASHAM, J. T. 1958. Decay of trembling aspen. Can. J. Botany, **36**, 491–505.
2. BLACK, R. L. and KRISTAPOVICH, P. J. 1954. Decay of trembling aspen in Manitoba and eastern Saskatchewan. Can. Dept. Agr., Forest Biol. Div., Saskatoon. Mimeographed.
3. FRITZ, C. W. 1954. Decay of poplar pulpwood in storage. Can. J. Botany, **32**, 799–817.
4. PAUL, G. and ETHERIDGE, D. E. 1958. Decay of aspen (*Populus tremuloides* Michx.) and balsam poplar (*P. balsamifera* L.) in the Lesser Slave Lake region in Alberta. Alberta Dept. Lands and Forests, Forest Survey Div., Edmonton, and Can. Dept. Agr., Forest Biol. Div., Calgary. Mimeographed.
5. RILEY, C. G. 1952. Studies in forest pathology. IX. *Fomes igniarius* decay of poplar. Can. J. Botany, **30**, 710–734.
6. ROWE, J. S. 1959. Forest regions of Canada. Can. Dept. Northern Affairs and Natl. Resources, Forest Serv., Ottawa. Bull. 123.
7. THOMAS, G. P. and PODMORE, D. G. 1953. Studies in forest pathology. XI. Decay in black cottonwood in the middle Fraser region, British Columbia. Can. J. Botany, **31**, 675–692.

INHIBITION OF RUST DEVELOPMENT ON DETACHED WHEAT LEAVES BY METABOLITES, ANTIMETABOLITES, AND ENZYME POISONS[1]

D. J. SAMBORSKI AND F. R. FORSYTH[2]

Abstract

The effect of metabolites and antimetabolites on rust development was studied, using detached leaves of Little Club wheat floated on solutions containing benzimidazole plus the compound under study. Purines and pyrimidines, vitamins, amino acids, carbohydrates, and enzyme poisons were tested. A number of these compounds inhibited leaf and stem rusts of wheat at concentrations that were not injurious to the host. Of the purines and pyrimidines that were tested, thymine and the analogue azathymine were the only effective inhibitors. The antivitamin oxythiamine was inhibitory and the inhibition was competitively reversed by thiamine.

A few natural amino acids, notably histidine, isoleucine, methionine, and serine, inhibited rust development. The inhibition was reversed by glycine in all cases except with serine. Amino acid analogues, particularly canavanine, ethionine, and *p*-fluorophenylalanine, were excellent inhibitors; the inhibitions were reversed by comparable levels of arginine, methionine, and phenylalanine respectively. The carbohydrates lyxose, xylose, sorbose, and all the sugar alcohols tested were effective inhibitors of rust development. Of the enzyme poisons tested, sodium fluoride and sodium azide differentially inhibited rust growth.

Introduction

Attempts have been made to correlate changes in rust reaction with changes in endogenous levels of substrate in the host (7, 20, 21). However, the experimental methods used invoke such profound disturbances in the host tissue that recognition of specific effects on the pathogen is difficult. More precise information on the importance to the host–parasite complex of specific compounds should be obtainable through the use of specific antimetabolites.

The usefulness of antimetabolites in the study of host–parasite relations may depend on the importance of simple metabolites and their metabolic products to rust development. In addition, it will be necessary to inhibit the pathogen without causing appreciable damage to the host. Differential sensitivity to a given antimetabolite can reasonably be expected. In fact, Hassebrauk (11, 12, 13) and Hotson (14) showed that rust development was inhibited by sulphonamides without injury to the host and that the inhibition was reversed by para-aminobenzoic acid. Increased resistance to *Venturia inaequalis* (Cke.) Wint. following injection of the host with analogues of normal metabolites has also been reported (15, 16). An interesting quantitative effect was reported by Badami, who found, working with tobacco, that the multiplication of tobacco mosaic virus was only slightly affected by azaguanine and almost prevented by thiouracil, whereas the multiplication of cucumber mosaic virus was greatly decreased by azaguanine and little affected by thiouracil (3).

[1]Manuscript received January 25, 1960.
Contribution No. 45 from the Canada Department of Agriculture Research Station, Winnipeg, Manitoba.
[2]Plant Pathology Laboratory.

Detached leaf culture, in the presence of benzimidazole, provides an excellent method for studying the action of antimetabolites (20, 26). The present paper describes the effect on rust development of some antimetabolites and a number of normal metabolites.

Methods

Little Club wheat, *Triticum compactum* Host., which is highly susceptible to both leaf rust, *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* (race 5*a*), and stem rust, *Puccinia graminis* (Pers.) f. sp. *tritici* Erikss. and Henn. (race 15B-1), was used exclusively as the susceptible host material. Khapli, *T. dicoccum* Schubler, was used as the resistant host in the carbohydrate-feeding experiment.

First leaves of 7- to 8-day-old wheat seedlings were detached 1 day after inoculation and floated on 20 ml of solution in petri dishes. Five leaves approximately two and one-half inches in length were used in each dish. All solutions contained benzimidazole (30 p.p.m. for leaf rust and 40 p.p.m. for stem rust) in addition to the compound under investigation.The petri dishes were kept in a growth chamber at 70° F, and illuminated for 16 hours each day at a light intensity of 700 ft-c.

Estimates of phytotoxicity were determined visually. Leaves on benzimidazole retain their chlorophyll during the experimental period (18, 20, 27) and any appreciable phytotoxicity is manifested by loss of chlorophyll. An additional estimate of phytotoxicity was obtained by studying respiratory drifts for wheat leaves floated on solutions which inhibited rust development without visual loss of chlorophyll. Leaves of Little Club wheat were floated on solutions of benzimidazole alone and on benzimidazole plus ethionine (100 p.p.m.), dulcitol (1%), and sodium azide (10^{-3} *M*). Samples were removed at 2-day intervals and the oxygen uptake was measured, using a Warburg apparatus. Five determinations were carried out over a 10-day period. Drifts of oxygen uptake for leaves floated on ethionine, dulcitol, and azide were the same as the drifts for leaves floated on benzimidazole alone. It appears that there is no appreciable phytotoxicity when chlorophyll is not being destroyed.

All biochemicals used in this study were obtained from Nutritional Biochemicals Company.

Results

Infected leaves treated with a series of concentrations of a particular compound show one of the following effects: (i) no phytotoxicity or rust inhibition up to the highest concentration tested; (ii) phytotoxicity and rust inhibition occurring at the same concentration, i.e., no differential effect; (iii) rust inhibition at concentrations which are not toxic to the host. A fourth category, phytotoxicity without rust inhibition, is possible but would not be readily detectable by the experimental methods used in this study. Studies on a group of purines and pyrimidines showed that uracil acted according to category

(i), adenine, azaadenine, cytosine, guanine, azaguanine, and 2,6-diaminopurine sulphate according to category (ii), while the effects of category (iii) were shown only by thymine and azathymine. Representative data are shown in Table I. Azathymine was effective at lower concentrations than was thymine.

TABLE I

Phytotoxicity of thymine, azathymine, and cytosine and effect on leaf rust development on detached leaves of Little Club wheat

Treatment (p.p.m.)		Phytotoxicity*	Rust development*
Thymine	2000	0	0
	1000	0	++++
Azathymine	1000	0	0
	500	0	+
	250	0	++
	125	0	++++
Cytosine	2000	++	+
	1000	0	++++

*In this and following tables 0 = none and ++++ = severe symptoms.

Several investigators have studied the effect of thiamine feeding on rust development. Pryor (19) observed that thiamine feeding led to improved mildew development on detached cantaloupe leaves. However, Gottlieb and Hart (10) found no effect of thiamine on cereal rusts. The data in Table II show that oxythiamine, an antithiamine, inhibited rust development and that the inhibition was completely reversed by thiamine. Undoubtedly thiamine feeding would improve rust development in situations where the thiamine level is suboptimal. While these results, and those of Hassebrauk (11, 12, 13) and Hotson (14), demonstrate the importance of vitamin metabolism to the host–parasite complex, they do not indicate whether the effects are related to a growth factor requirement on the part of the pathogen, or to an alteration in the metabolism of the host.

TABLE II

Phytotoxicity and inhibition of leaf rust development on detached wheat leaves by oxythiamine and the reversal of this inhibition by thiamine

Treatment (p.p.m.)		Phytotoxicity	Rust development
Oxythiamine	1000	++	0
	500	+	0
	250	0	0
	125	0	+
	62.5	0	++
Oxythiamine 250		0	0
Oxythiamine 250 + thiamine	62.5	0	++
Oxythiamine 250 + thiamine	125	0	++
Oxythiamine 250 + thiamine	250	0	++++

A number of amino acids and amino acid analogues were also tested. The effects of these on detached leaves, and on rust development, are grouped and shown in Table III. Leaf and stem rusts of wheat reacted similarly, and were inhibited by a number of these compounds. The inhibition of rust

development by certain natural amino acids is particularly interesting since competitive inhibition is well known in microbiology and the inhibitions can be reversed by other amino acids (28). The data in Table IV show that glycine reversed the inhibitory effect of histidine and methionine but not of serine. The amino acid interactions have not been examined in detail but probably other amino acids would be effective reversal agents and indeed, glycine may be a secondary reversing agent.

TABLE III

A classification of some amino acids and amino acid analogues at concentrations up to 0.2% on the basis of phytotoxicity and rust inhibition

No phytotoxicity or rust inhibition	Rust inhibition and phytotoxicity at same levels	Slight to moderate rust inhibition at non-phytotoxic levels	Rust completely inhibited at non-phytotoxic levels
	Amino acids		
Alanine	Aspartic acid	Arginine	Histidine
Glycine	Glutathione	Citrulline	Isoleucine
Leucine	Hydroxyproline	Cysteine	Methionine
Lysine	Ornithine	Glutamic acid	Serine
Valine	Phenylalanine	Tryptophane	
	Proline	Threonine	
	Tyrosine		
	Amino acid analogues		
	Norleucine	5-Carbonyl-cysteine	Canavanine
	Norvaline	5-Methyl-tryptophan	Ethionine
	B-2-Thienylalanine		*p*-Fluorophenyl-alanine
	B-2-Thienylserine		

TABLE IV

Inhibition of leaf rust development on detached wheat leaves by natural amino acids and the effect of glycine on this inhibition

Treatment		Rust development	
		Single amino acid	Plus glycine (0.2%)
DL-Serine	(0.2%)	0	0
DL-Histidine	(0.2%)	0	++++
L-Methionine	(0.1%)	0	++++
Glycine	(0.2%)	++++	++++

Canavanine, ethionine, and *p*-fluorophenylalanine were the most effective at inhibiting rust of the amino acid analogues tested. The data in Table V show that the lowest effective concentration was different for each analogue. Partial or complete reversal of inhibition was obtained in each case by the addition of the appropriate metabolite (Table VI). Glycine at considerably higher levels also reversed, at least partially, the inhibitory effects of these analogues. In this situation, glycine was undoubtedly acting as a secondary reversing agent. However, it should be noted that the inhibitory action of ethionine was most effectively reversed by methionine plus glycine.

TABLE V

Phytotoxicity of amino acid analogues and effect on leaf rust development on detached leaves of Little Club wheat

Treatment (p.p.m.)		Phytotoxicity	Rust development
Canavanine	100	++++	0
	10	0	0
	5	0	++
	1	0	+++
	0.1	0	++++
p-Fluorophenylalanine	200	0	0
	100	0	+
	50	0	++
	25	0	++++
Ethionine	400	0	0
	200	0	0
	100	0	0
	50	0	+

TABLE VI

Inhibition of leaf rust development on detached wheat leaves by amino acid analogues and the reversal of inhibition by natural amino acids

Treatment (p.p.m.)				Rust development
Canavanine	10			0
	+ arginine		50	+
	+ arginine		100	+++
	+ arginine		200	+++
	+ glycine		100	0
	+ glycine		500	++
	+ glycine		1000	+++
Ethionine	100			0
	+ methionine		50	+
	+ methionine		100	++
	+ glycine		100	0
	+ glycine		500	0
	+ glycine		1000	+
	+ methionine	100 + glycine	100	++++
p-Fluorophenylalanine	200			0
	+ phenylalanine		150	+
	+ phenylalanine		300	++
	+ phenylalanine		600	++++
	+ glycine		250	+
	+ glycine		500	++
	+ glycine		1000	++

Many investigators have studied the effect of carbohydrates on rust development (see refs. 1, 29). A number of carbohydrates support the growth of rusts in detached leaves of susceptible varieties and an exogenous supply of carbohydrate may change a resistant to a susceptible reaction. However, Sempio (22) reported that sorbose prevented rust development without injuring detached bean leaves. The data in Table VII show that sorbose was equally effective in preventing rust development in detached wheat leaves. Other

carbohydrates were also very inhibitory to the rusts. Methyl pentoses, hexoses (other than sorbose), disaccharides, and trisaccharides permitted abundant rust development while some pentoses and all of the sugar alcohols tested were inhibitory.

TABLE VII

Phytotoxicity of carbohydrates and effect on stem rust development on detached leaves of resistant Khapli and susceptible Little Club wheats

Treatment (1%)	Phytotoxicity*	Rust development	
		Khapli	Little Club
Pentoses			
L-Arabinose	0	+	++++
D-Arabinose	+	+	+
D-Lyxose	0	0	0
D-Ribose	0	++	+++
D-Xylose	0	+	+
Methyl pentoses			
L-Rhamnose	0	++	++++
L-Fucose	0	++	++++
Hexoses			
D-Fructose	0	+++	++++
D-Glucose	0	+++	++++
D-Galactose	0	+++	++++
D-Mannose	++	—	—
L-Sorbose	0	0	0
Disaccharides			
D-Cellobiose	0	+++	++++
B-Lactose	0	+++	++++
D-Maltose	0	++	+++
D-Melobiose	0	+++	++++
D-Sucrose	0	+++	++++
D-Trehalose	0	+++	++++
Trisaccharides			
D-Melizitose	0	+++	++++
D-Raffinose	0	+++	++++
Sugar alcohols			
Adonitol	0	0	0
Arabitol	0	0	0
Dulcitol	0	0	0
Erythritol	0	0	0
Mannitol	0	0	0
Sorbitol	0	0	0

*Phytotoxic effects were the same for both varieties of wheat.

Dulcitol was the most effective of the carbohydrates tested as an inhibitor of rust development (Table VIII). Little Club leaves were detached 1, 3, and 5 days after inoculation and floated on 1% dulcitol. Rust development was arrested and no further growth occurred in the presence of dulcitol. Inhibition and blackening of stem rust pustules in the presence of sugar alcohols has been observed also by Silverman (23).

No information was obtained on the mechanisms by which carbohydrates may inhibit rust development. The inhibitory action of dulcitol and sorbose

could not be reversed by the addition of glucose or galactose. Possibly they act through altering the host metabolism since dulcitol had no effect on rust spore germination and germ tube elongation.

TABLE VIII

Effect of concentration of selected carbohydrates on stem rust development on detached leaves of Little Club wheat

	Concentration (%)			
	1	0.5	0.25	0.125
Carbohydrate	Rust development			
Ribose	+++	++++	++++	++++
Xylose	+	++++	++++	++++
Erythritol	0	+++	++++	++++
Sorbose	0	0	++++	++++
Dulcitol	0	0	0	0

A number of enzyme inhibitors were tested. Sodium fluoride and sodium azide inhibited rust development at non-phytotoxic concentrations while sodium malonate, sodium diethyldithiocarbamate, iodoacetate, *o*-phenanthroline, and 2,4-dinitrophenol generally resulted in toxicity to both host and parasite. Representative data, to illustrate the non-differential effects of malonate and iodoacetate, are given in Table IX. Data for several experiments with fluoride and azide are shown in Table X; here it may be seen that although strikingly different results were obtained from the same treatments in different experiments, in any single experiment the pathogen was inhibited at levels of treatment that were non-phytotoxic.

TABLE IX

Phytotoxicity of sodium malonate and iodoacetate and their effects on leaf rust development on detached wheat leaves

Treatment	Phytotoxicity	Rust development
Sodium malonate		
10^{-2} *M*	++	++
5×10^{-3} *M*	+	++++
10^{-3} *M*	0	++++
Iodoacetate		
5×10^{-3} *M*	+++	0
10^{-3} *M*	++	0
5×10^{-4} *M*	+	++
10^{-4} *M*	0	+++

Shu, Tanner, and Ledingham (25) found that fluoride had no effect on the respiration of rust spores which apparently metabolize carbohydrates via the pentose phosphate pathway (24). Therefore, it is probable that the inhibitory action of fluoride is indirect through its effect on carbohydrate metabolism in the host. The effect of azide is most likely a direct one on the fungus since Allen and Goddard (2) found that azide would differentially inhibit mildew respiration without affecting wheat respiration.

TABLE X

Phytotoxicity of sodium fluoride and sodium azide and their effects on leaf rust development on detached wheat leaves

Inhibitor		Concentration (M)	Phytotoxicity	Rust development
NaF	Expt. 1	10^{-4}	0	++
		2.5×10^{-4}	0	+
		5×10^{-4}	0	0
		7.5×10^{-4}	0	0
		10^{-3}	0	0
	Expt. 2	5×10^{-4}	0	++
		7.5×10^{-4}	0	+
		10^{-3}	0	0
	Expt. 3	10^{-3}	0	++
		5×10^{-2}	0	0
		10^{-2}	+++	0
NaN_3	Expt. 1	10^{-4}	0	++++
		2.5×10^{-4}	0	+++
		5×10^{-4}	0	++
		7.5×10^{-4}	0	+
		10^{-3}	0	0
	Expt. 2	10^{-4}	0	0
		5×10^{-4}	+++	0
		10^{-3}	++++	0
	Expt. 3	10^{-4}	0	++++
		5×10^{-4}	0	+
		10^{-3}	++	+

Discussion

Most of the compounds tested in this study affect reactions that are known to occur in the host. It is therefore surprising that so large a proportion of those tested inhibited rust development without showing any apparent injury to the host. This difference in sensitivity could occur if a given antimetabolite can temporarily interrupt a particular synthesis that is vital to the fungus, but relatively unimportant to the mature cells of the host. In addition, the tissue of the parasite may be more permeable to an inhibitor than is the host tissue.

There is considerable evidence that some antimetabolites act by preventing the metabolite from entering the cell rather than by inhibiting its utilization (28). Other antimetabolites compete with the metabolite for particular enzymes or templates. Further information is needed to determine the mode of action of the antimetabolites used in this study. However, the inhibition of rust development by natural amino acids may be similar to that which is observed with bacteria. Cohen and Rickenberg (6) found that the competitive inhibition of bacterial growth by natural amino acids—related in structure to other natural amino acids—was satisfactorily explained in terms of competition for specific permeases. They define permease as being a system of a protein nature catalyzing the transfer of a substrate across a cellular osmotic barrier, possessing the properties of steric specificity and kinetic activity of an enzyme, but distinct and independent of enzymes bringing about the metabolism as such of the substrate. Cohen (5) found that inhibition of growth by valine in *E. coli* was associated with the synthesis of abnormal proteins which

were high in valine and lacked enzymatic activity. Clarke and Meadow (4) concluded that permeases function in the uptake of tricarboxylic-acid-cycle intermediates in *Pseudomonas aeruginosa*. They stated that substrates of the oxidative system induce the formation of their own permeases.

Mathieson and Catcheside (17) found that the toxicity of arginine and methionine to a histidine-requiring mutant of *Neurospora crassa* was due to the inhibition of histidine uptake by arginine and methionine. This system appears to be analogous to that of *E. coli* but with less strict specificity. There is no information on the possible role of permeases in host–parasite relations but the inhibition of rust growth by natural amino acids (Table IV) probably reflects abnormal amino acid uptake by the fungal cell. Fuchs and Bauermeister reported that inhibition of stem rust development by thiosemicarbazide appeared to result from changes in the metabolism of the host (8). An outstanding effect of thiosemicarbazide on wheat plants was the marked increase in free amino acids. It is possible that inhibition of the rust was due to excessive levels of certain amino acids.

It has been suggested than an adequate level of essential amino acids is required for normal rust development (20). The present results suggest further that the effective concentrations of an amino acid must lie within certain limits relative to those of other amino acids. An amino acid may stimulate growth at one concentration and be inhibitory at another. An example can be drawn from the results with ethionine and methionine. Methionine at 100 p.p.m. overcame the inhibitory effect of 100 p.p.m. ethionine while at 1000 p.p.m. methionine itself was inhibitory. A certain level of methionine is essential to the host–parasite complex but an excessive level, relative to other amino acids, is inhibitory. Inhibition–nutrition phenomena in host–parasite relations have been discussed by Garber (9). The experiments reported in this paper were largely exploratory, to determine the usefulness of feeding techniques in studying host-parasite relations. The results have been encouraging and further studies, particularly on the mechanism of action of the inhibitory compounds, are planned.

References

1. ALLEN, P. J. 1954. Physiological aspects of fungus diseases of plants. Ann. Rev. Plant Physiol. **5**, 225–248.
2. ALLEN, P. J. and GODDARD, D. R. 1938. A respiratory study of powdery mildew of wheat. Am. J. Botany, **25**, 613–621.
3. BADAMI, R. S. 1959. Some effects of changing temperature and of virus inhibitors on infection by cucumber mosaic virus. Ann. Appl. Biol. **47**, 78–89.
4. CLARKE, P. H. and MEADOW, P. M. 1959. Evidence for the occurrence of permeases for tricarboxylic acid cycle intermediates in *Pseudomonas aeruginosa*. J. Gen. Microbiol. **20**, 144–155.
5. COHEN, G. N. 1958. Synthèse de protéines "anormales" chez *Escherichia coli* K12 cultivé en présence de L-valine. Ann. inst. Pasteur, **94**, 15–30.
6. COHEN, G. N. and RICKENBERG, H. V. 1956. Concentration spécifique réversible des amino acids chez *Escherichia coli*. Ann. inst. Pasteur, **91**, 693–720.
7. FORSYTH, F. R. and SAMBORSKI, D. J. 1958. The effect of various methods of breaking resistance on stem rust reaction and content of soluble carbohydrate and nitrogen in wheat leaves. Can. J. Botany, **36**, 717–723.
8. FUCHS, W. H. and BAUERMEISTER, R. 1958. Uber die wirkung von thiosemicarbazid auf die entwicklung von *Puccinia graminis tritici* auf weizen. Naturwissenschaften, **45**, 343–344.

9. GARBER, E. D. 1956. A nutrition–inhibition hypothesis of pathogenicity. Am. Naturalist, **90**, 183–194.
10. GOTTLIEB, D. and HART, H. 1943. Growth substances and the rust fungi. Phytopathology, **33**, 724–728.
11. HASSEBRAUK, K. 1951. Untersuchungen über die einwirkung von sulfonamiden und sulfonen auf getreideroste. I. Die beeinflussing des fruktifikationsvermögens. Phytopathol. Z. **17**, 384–400.
12. HASSEBRAUK, K. 1952. Untersuchungen über die einwirkung von sulfonamiden und sulfonen auf getreideroste. II. Weitere untersuchungen über die rosthemmende wirkung. Phytopathol. Z. **18**, 453–460.
13. HASSEBRAUK, K. 1952. Untersuchungen über die einwirkung von sulfonamiden und sulfonen auf getreideroste. III. Untersuchungen über den wirkungsmechanismus von sulfonamiden und sulfonen. Phytopathol. Z. **19**, 56–78.
14. HOTSON, H. H. 1952. The inhibition of wheat stem rust by sulfadiazine and the reversal of this effect by para-amino benzoic acid and folic acid. (Abstr.) Phytopathology, **42**, 11.
15. KIRKHAM, D. S. and FLOOD, A. E. 1956. Inhibition of *Venturia* spp. by analogues of host metabolites. Nature, **178**, 422–423.
16. KUC, J., BARNES, E., DAFTSIOS, A., and WILLIAMS, E. B. 1959. The effect of amino acids on susceptibility of apple varieties to scab. Phytopathology, **49**, 313–315.
17. MATHIESON, J. M. and CATCHESIDE, G. O. 1955. Inhibition of histidine uptake in *Neurospora crassa*. J. Gen. Microbiol. **13**, 72–83.
18. PERSON, C., SAMBORSKI, D. J., and FORSYTH, F. R. 1957. Effect of benzimidazole on detached wheat leaves. Nature, **180**, 1294–1295.
19. PRYOR, D. E. 1942. The influence of vitamin B_1 on the development of cantaloupe powdery mildew. Phytopathology, **32**, 885–895.
20. SAMBORSKI, D. J., FORSYTH, F. R., and PERSON, C. 1958. Metabolic changes in detached wheat leaves floated on benzimidazole and the effect of these changes on rust reaction. Can. J. Botany, **36**, 591–601.
21. SAMBORSKI, D. J. and SHAW, M. 1957. The physiology of host–parasite relations. IV. The effect of maleic hydrazide and indoleacetic acid on the rust resistance of Khapli and Little Club wheats. Can. J. Botany, **35**, 449–455.
22. SEMPIO, C. 1942. Influenza di alcuni glucidi isomeri sullo sviluppo della ruggine del fagiolo e di altre malattie fungine. Riv. biol. (Perugia), **34**, 52–56.
23. SILVERMAN, W. B. 1957. The development of *Puccinia graminis* var. *tritici* on detached leaves of wheat as influenced by nutrients and metabolite antagonists. (Abstr.) Phytopathology, **47**, 31–32.
24. SHU, P. and LEDINGHAM, G. A. 1956. Enzymes related to carbohydrate metabolism in uredospores of wheat stem rust. Can. J. Microbiol. **34**, 489–495.
25. SHU, P., TANNER, K. G., and LEDINGHAM, G. A. 1954. Studies on the respiration of resting and germinating uredospores of wheat stem rust. Can. J. Botany, **32**, 16–23.
26. WANG, D. 1959. Effect of benzimidazole, dimethylbenzimidazole, glucose, and metal ions on the development of *Puccinia graminis tritici* on detached leaves of Khapli wheat. Can. J. Botany, **37**, 239–244.
27. WANG, D. and WAYGOOD, E. R. 1959. Effect of benzimidazole and nickel on the chlorophyll metabolism of detached leaves of Khapli wheat. Can. J. Botany, **37**, 743–749.
28. WOODS, D. D. and TUCKER, R. G. 1958. The relation of strategy to tactics: Some general biochemical principles. *In* The strategy of chemotherapy. Symposium Soc. Gen. Microbiol. No. 8. Cambridge University Press.
29. YARWOOD, C. E. 1946. Detached leaf culture. Botan. Rev. **12**, 1–56.

THE INFLUENCE OF SMELTER FUMES UPON THE CHEMICAL COMPOSITION OF LAKE WATERS NEAR SUDBURY, ONTARIO, AND UPON THE SURROUNDING VEGETATION[1]

EVILLE GORHAM AND ALAN G. GORDON

Abstract

Analyses for sulphate, calcium, and pH have been made on surface waters from 102 lakes and ponds in the Sudbury metal-smelting district, and data are presented for 35 of these. Sulphur pollution is frequently high within about 5 miles of the three smelters, many ponds exhibiting more than three times the sulphate concentration normal for this area, and three waters more than 10 times this level. Outside about 15 miles distance the influence of smelter pollution upon sulphate concentrations in surface waters is negligible. As expected, many of the most polluted waters are strongly acid, with pH values going as low as 3.3. Sulphuric acid from air pollution has also led to increased weathering of calcium from soils and rocks, so that this ion tends to rise in concentration not only in waters above pH 6 (as expected) but also in those below pH 5.

Damage to terrestrial vegetation is frequently marked within about 5 miles of the smelters, while it is seldom obvious to the untrained eye beyond this distance. Severe damage occurs chiefly within about 2 miles of the smelters.

Introduction

In the vicinity of Sudbury, nickel and copper smelting at Copper Cliff, Coniston, and Falconbridge results in emission to the atmosphere of roughly one million tons of sulphur per annum, chiefly as sulphur dioxide (1). Damage to vegetation is severe close by the smelters, and the occasional fumigations which occur under special weather conditions may affect sensitive plants such as white pine at least as far as 25 miles away (8).

Although emission of sulphur dioxide is monitored by a series of automatic recorders in nine widely separated locations (2), the fall-out pattern is not known in any detail. The present investigation was undertaken to provide information upon this point, to ascertain something of the influence of smelter pollution on lake waters in the area, and to examine the effects upon vegetation near the waters surveyed. A general helicopter survey of damage to terrestrial vegetation was also attempted.

Methods

During June 1959, 102 surface water samples were collected by helicopter and automobile from a wide variety of sites in the Sudbury area, in most cases from small and relatively undisturbed bodies of water with restricted local drainage. The samples were taken in pint polyethylene bottles, which were transported to Toronto for analysis within the next month.

Since sulphur dioxide from the smelters is eventually oxidized to sulphate, this anion was chosen as an index of fall-out from such sources, and was measured by the technique of Mackereth (9). (Nitrate is included with sulphate by this method, but is likely to be present in these waters only in extremely

[1]Manuscript received February 10, 1960.
Contribution from the Botany Department, University of Toronto, and the Research Branch, Ontario Department of Lands and Forests.

TABLE I

Representative analyses of waters from the Sudbury area

	Sample No.	Miles from nearest smelter*	Direction from smelter	SO_4, meq per l.	pH		Ca, meq per l.
					Before aeration	After aeration	
(*a*) Lake Wanapitei	42	ca. 12 F	NNE.	0.34	7.23	7.47	0.43
(*b*) Waters more than 15 miles from smelters	95	19.4 F	NNE.	0.37	4.40	4.39	0.17
	29	16.4 CC	S.	0.36	4.60	4.62	0.21
	92	16.6 CC	N.	0.30	4.79	4.90	0.13
	101	39 F	ENE.	0.31	5.00	5.05	0.14
	96	15.6 F	NNE.	0.47	5.34	5.59	0.26
	94	19.8 CC	WNW.	0.24	5.76	6.00	0.18
	102	35 F	NE.	0.29	5.73	6.04	0.17
	93	15.4 CC	NNW.	0.31	6.45	6.61	0.25
	97	15.5 F	NW.	0.31	6.20	6.70	0.22
	26	19.2 CC	SW.	0.24	6.70	7.18	0.27
	28	24.2 CC	SW.	0.39	7.11	7.39	0.41
	27	23.4 CC	SW.	0.29	7.28	7.41	0.34
Arithmetic average		21.6		0.32	5.78	5.99	0.23
(*c*) Dark-brown waters with heavy ferric hydroxide deposit (see also Nos. 41 and 87)	76	10.8 C	SE.	0.34	6.12	6.62	0.22
	80	3.3 F	ENE.	0.28	6.26	7.25	0.45
	85	2.2 F	WNW.	0.28	6.10	6.94	0.37
(*d*) Pond on silt–clay plain	87	6.4 F	WNW.	1.78	6.15	6.72	1.51
(*e*) Three most alkaline waters from kettle-holes in sandy outwash	17	4.0 F	NNE.	0.60	7.67	7.90	0.98
	18	4.1 F	NNE.	0.60	7.43	7.93	1.42
	40	5.2 F	NNE.	0.66	7.34	7.90	1.12
(*f*) Pond receiving much eroded soil	65	1.8 C	SSW.	1.88	5.76	6.10	1.04
(*g*) Examples of severe pollution by atmospheric sulphur compounds	70	1.5 C	NNE.	6.92	3.40	3.40	0.84
	46	1.8 CC	NE.	6.18	3.46	3.45	1.20
	57	1.3 CC	S.	4.35	3.61	3.61	0.58
	7	0.7 F	S.	3.53	3.49	3.50	0.68
	73	4.5 CC	ENE.	2.84	3.51	3.51	0.69
	8	0.7 F	S.	2.75	3.23	3.26	0.70
(*h*) Examples of other human interference							
Sewage	58	1.7 CC	S.	7.07	4.55	4.56	3.55
Railway fill, possible mine waste too	52	2.0 CC	NNW.	10.00	3.56	3.56	4.36
Pond near Garson School, mine waste	5	3.2 F	WSW.	5.00	7.30	7.91	4.78
Some drainage from smelter sludge dump	59	3.4 CC	WSW.	3.42	4.16	4.16	1.19
Clay pit	67	2.2 C	E.	1.12	5.82	6.46	0.65
Silty sand pit	38	4.8 F	WSW.	0.69	7.60	7.98	1.33
Gravel pit	91	11.2 F	NW.	0.44	6.41	6.64	0.83
Pond banked with sawdust	41	7.4 F	NNE.	0.66	5.86	7.00	0.53

*CC—Smelter at Copper Cliff, C—Smelter at Coniston, F—Smelter at Falconbridge.

low concentrations as compared with sulphate.) Because oxidation of sulphur dioxide commonly produces sulphuric acid, the pH values of the water samples were estimated by glass electrode, both before and after aeration to drive off excess carbon dioxide. As the lime status of the soil is also likely to affect the pH of water draining it, calcium concentrations were determined by EDTA titration, with ammonium purpurate as indicator (7). Potassium cyanide was employed in the routine to complex heavy metals which interfered with calcium analyses in waters collected near the smelters. Qualitative visual estimations of water color, and of ferric hydroxide deposition on standing, were also made.

Damage to terrestrial vegetation in the vicinity of the lakes was noted subjectively as very severe, severe, considerable, moderate, or not obvious, on brief examination from some distance away. The categories are defined in Table II. Estimates of the boundaries between the various categories were later made from the air on a series of helicopter transects, and these were used to check the general validity of the lakeside observations. It should be remarked that the estimates of damage refer only to vegetation on thin soils in relatively exposed situations; on the deeper soils in protected valleys damage is not nearly so great.

Results of the Water Analyses

Data for 35 representative water samples are presented in Table I. In addition all the sulphate analyses are shown in Fig. 1. Some waters which have been subjected to human interference apart from smelter pollution are indicated separately as crosses on this figure.

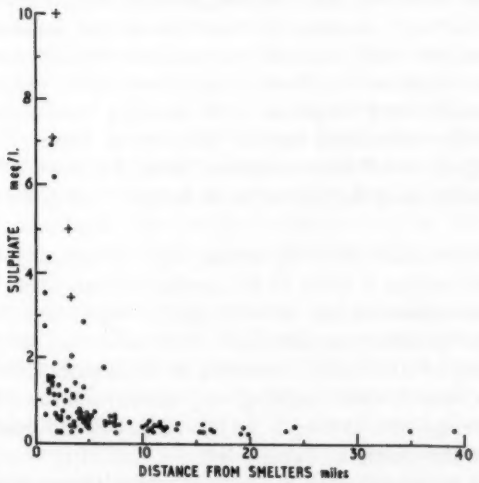


FIG. 1. Sulphate concentrations in lakes and ponds near Sudbury. Crosses represent ponds subject to other than aerial sulphur pollution.

Sulphate

Figure 1 shows very clearly the influence of air pollution in raising concentrations of sulphate in pond and lake waters near the smelter stacks. The maximum level reached in any water not otherwise subject to human interference was 6.92 meq/l., recorded for a small shallow pool on a rocky knoll 1.5 miles NNE. of the Coniston smelter (Table I (*g*), No. 70). The water had probably been concentrated a good deal by evaporation. At this site there is no living higher vegetation whatever, although a few red maple and red oak bushes are present with a small number of associated species in less exposed situations about 100 yards away. A second sample, 1.8 miles NE. of Copper Cliff (Table I (*g*), No. 46), had almost as much sulphate, 6.18 meq/l. The vegetation here is again sparse, with red maple bushes predominating. The next highest of such values was 4.35 meq/l., from another similar pond (Table I (*g*), No. 57) about 1.3 miles S. of the Copper Cliff smelter, with only a few red maple bushes growing around the site.

Of the three other water samples highest in sulphate, all of which contained 5 meq/l. or more, one (Table I (*h*), No. 5) was taken from a pond which drains large amounts of mine waste rich in sulphur. A second (Table I (*h*), No. 52) has had abundant railway fill dumped in it, quite possibly from a mine nearby. The third such sample came from Kelley Lake, about 1.7 miles S. of Copper Cliff (Table I (*h*), No. 58). This lake receives very large amounts of sewage from the Sudbury area.

All but one of the 32 waters with sulphate levels above 1 meq/l. were collected within 5 miles of a smelter stack, the exception being a small pond (Table I (*d*)) about 6.4 miles WNW. of Falconbridge, with water containing 1.78 meq/l. This pond was the only one observed on the extensive silt–clay plain north of Sudbury; presumably these heavy and low-lying agricultural soils are comparatively rich in sulphur compounds. One other sample (Table I (*g*), No. 73), not quite so far from the smelters as the preceding one, also exhibited unusually high sulphate (2.84 meq/l.), without any apparent possibility of other than aerial supply. The site is, however, very centrally located with regard to all three smelters, being 4.5 miles ENE. of Copper Cliff, and no doubt owes its high sulphate levels to air pollution from all of them.

Neglecting the waters receiving other than air-borne pollution, the 49 samples collected within 5 miles of the smelter stacks yielded a logarithmic average sulphate concentration of 0.94 meq/l., logarithms being employed to normalize the frequency distribution. This value may be compared with the concentration of 0.34 meq/l. recorded for Wanapitei Lake (Table I (*a*)), which is a large body of water draining very extensive areas NE. of Sudbury, and with the average of 0.32 meq/l. for the 12 waters collected more than 15 miles away from the smelters (Table I (*b*)).

It should also be remarked that only four of the 49 waters taken within 5 miles of the smelters gave sulphate concentrations of less than 0.40 meq/l.

Three of these samples (for two of them see Table I (*c*), Nos. 80 and 85) were strongly colored by brown organic compounds, and also deposited considerable reddish-brown ferric hydroxide on standing; microbiological reduction may be presumed to account for the low levels of sulphate in these waters.

In general it may be said that Fig. 1 shows a pronounced fall-out of sulphur within 5 miles of the smelters, and only slight fall-out farther away, the smelter effects becoming negligible beyond about 15 miles. It must of course be remembered that occasional severe fumigations occur beyond this distance, and may have considerable biological significance even though they are insufficient to affect lake water concentrations of sulphate.

Acidity

The pH range in unaerated water was 3.23–7.67; after aeration to remove excess carbon dioxide the range became 3.26–8.03. Wanapitei Lake (Table I (*a*)) exhibited values of 7.23 (original) and 7.47 (aerated). Below pH 4.5 the increase following aeration was probably within the range of error, while between 4.5 and 5.0 it was seldom more than 0.1 units. Above this limit the influence of dissolved carbon dioxide increased markedly, and throughout the original pH range of 5.4–7.7, aeration resulted in a rise of about 0.15–0.85 units. Two exceptional samples (Table I (*c*), No. 80, and (*h*), No. 41) showed even greater increases of pH upon aeration, amounting to 0.99 and 1.14 units respectively. In both these pond waters there was a strong brown humus coloration, with in addition a heavy deposit of ferric hydroxide on standing; indeed, many of the waters exhibiting the most marked pH increases upon aeration showed such iron deposits. Presumably most of the iron derived from ferrous bicarbonate, which upon oxidation would release considerable carbon dioxide into the water, and thus lower pH below the levels characteristic of the other waters.

While carbonic acid was present in most of these waters, the strongly acid samples undoubtedly owed their low pH values to free sulphuric acid, with all nine samples of pH less than 3.7 showing sulphate concentrations above 1.3 meq/l. Apart from some of these obviously polluted waters, the highest equivalent ratio of hydrogen ions to sulphate ions (0.36) came from a small pool set in an extensive area of drained bog peat 4.9 miles N. of Copper Cliff. This pool contained water with a sulphate level of 0.55 meq/l. and a pH of 3.70, the ionic ratio of hydrogen to sulphate being not unusual for bog waters.

The general trend in these Sudbury waters was for sulphate to be relatively low, about 0.25–0.75 meq/l., above pH 5; with a gradual rise to about 0.5–1.5 meq/l. as pH fell to 4, and a very sharp rise below pH 4 (see Table I (*g*) for the most acid samples). However, in five circumneutral waters between pH 6 and 7, sulphate concentrations of 1–2 meq/l. were observed. With the exception of the sample from the silt–clay plain these waters were collected from ponds fairly near the smelters and in sites exhibiting considerable soil erosion, the most extreme example being a water collected 1.8 miles SSW.

of Coniston (Table I (*f*)). Such erosion, brought about mainly by pollution damage to the vegetation, doubtless accounts for the low acidity of these waters, since it enriches them in lime. For instance, the water mentioned above (Table I (*f*)) had a calcium concentration of 1.04 meq/l.

One single pond, near Garson School and about 3.2 miles WSW. of Falconbridge (Table I (*h*), No. 5), combined high sulphate with an alkaline reaction; this pond drains spoil heaps near the Garson mine. In contrast, another pond which contains a great deal of railroad fill combined high sulphate with strong acidity (see Table I (*h*), No. 52). Relative to its high sulphate concentration, Kelley Lake (Table I (*h*), No. 58) was not very acid, perhaps owing to the inflow of large amounts of sewage.

It is noteworthy that even far from the smelters some pond waters were moderately acid, probably owing to the presence of sulphuric acid from natural sources (3, 4, 5). For example three waters at 10, 12, and 19 miles distance (for one of these see Table I (*b*), No. 95) exhibited pH values of 4.3–4.4, with about 0.4 meq of sulphate per liter. All these waters were low in calcium, which only amounted to 0.15–0.17 meq/l.

Calcium

The waters richest in calcium were the pond near Garson School (Table I (*h*), No. 5) with 4.78 meq/l., a pond 2 miles NNW. of Copper Cliff (Table I (*h*), No. 52) with 4.36 meq/l., and Kelley Lake (Table I (*h*), No. 58) with 3.55 meq/l. The first drains mine waste, and the second contains abundant railway fill, possibly from a mine nearby. Kelley Lake, on the other hand, is a major receiver of Sudbury sewage. Of the less disturbed ponds, that on the silt–clay plain (Table I (*d*)) had water containing 1.51 meq of calcium per liter, while several rather alkaline waters from among the ponds located in sandy outwash north and east of Falconbridge were also fairly rich in calcium, with up to 1.42 meq/l. (for the three most alkaline samples see Table I (*e*)). Of the ponds with waters between pH 6 and 7, those into which there has been considerable erosion were relatively high in calcium, and reached a maximum of 1.04 meq/l. (see Table I (*f*)). Between pH 5 and 6 calcium levels were less than 0.4 meq/l., while below pH 5 they tended to rise again, probably because the sulphuric acid produced from smelter fumes weathers additional lime from the soil. Among the acid waters affected only by the smelter fumes and not by fill or waste of any kind, the maximum calcium concentration observed was 1.2 meq/l. (Table I (*g*), No. 46). It was noticeable that among such acid waters, those from the more peaty drainage areas tended to exhibit higher calcium levels at a given pH, possibly because a lesser concentration of acid is required to bring about replacement of calcium adsorbed by peat than to weather it from inorganic soil minerals. The lowest calcium value found was 0.06 meq/l. in a sample of pH 3.9; apart from this several samples between pH 3.7 and 5.4 yielded concentrations of about 0.15 meq/l. Water from Wanapitei Lake (Table I (*a*)), collecting drainage over a wide area to the north and east of Sudbury, contained 0.43 meq/l.

TABLE II

Approximate categories of pollution damage used in the survey of terrestrial vegetation

	Damage				
	Not obvious	Moderate	Considerable	Severe	Very severe
Vegetation cover*	Complete	Up to ¼ knolls exposed	¼ to ½ of rock and shallow soil exposed	Over ½ of surface exposed	Whole surface exposed
Crown damage†	Not evident except for chlorosis on white pine	On ¼ trees, esp. birch; some spp. chlorotic	Slight to moderate on all trees, or considerable on more than ¼ trees	Extensive on most and apparent on nearly all trees. Suckering from base common	Extensive on few remaining trees. Suckering from base prevalent.
Species present					
Red maple	+	+	+	+	+
Red oak	+	+	+	+	
White birch	+	+	+	+	
Red pine	+	+	+		
Jack pine	+	+	+		
White spruce	+	+			
Trembling aspen	+	+			
Large-toothed aspen	+	+			
White pine	+				
Soil erosion	None	Not well marked	Evident	Strongly evident	Organic horizon almost wholly gone

*May be more or less evenly distributed, or in mosaic patterns of variable damage.
†Leads from chlorosis to browning of foliage, death of whole twigs, and in severe cases to extensive stem dieback.

Survey of Pollution Damage to Terrestrial Vegetation

The notes on vegetation damage near the ponds were usually made from the helicopter while taking water samples at a short distance from shore. Although qualitative (see Table II for definition of categories, and Fig. 2 for illustrations of them), their value is increased by the fact that a great number of sites was examined in all directions from the sources of pollution, and it may be added that local impressions were largely verified later by aerial survey during a special series of helicopter flights.

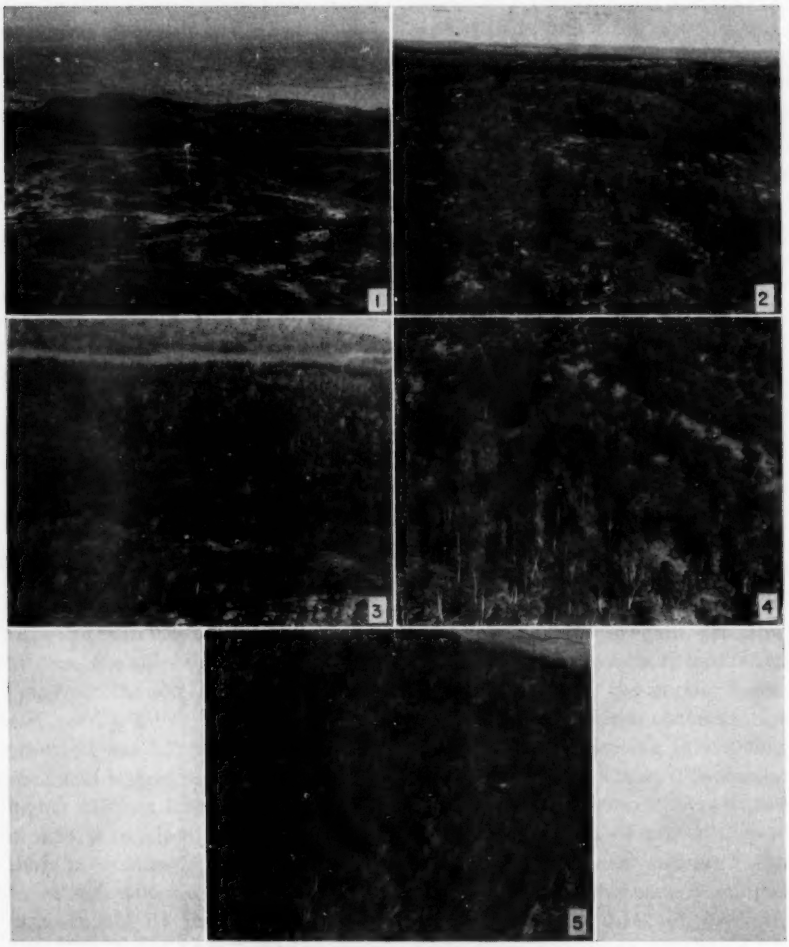


FIG. 2. Examples of the different categories of vegetation damage used in this survey: (1) very severe, (2) severe, (3) considerable, (4) moderate, (5) not obvious.

Table III summarizes the local notes, which refer only to thin soils in sites well exposed to pollution. It indicates that extensive damage is generally restricted to the area within 5 miles of the smelters, that is, the same area within which extremely high levels of sulphur fall-out were observed. Moreover, the very severely damaged sites were all located within 2 miles of the smelters. Beyond 5 miles damage to terrestrial vegetation was observed only twice, although some species such as white pine were absent beyond this distance owing to their especial sensitivity (cf. refs. 6, 8).

Regarding the later aerial observations, these suggested that the boundary between very severe and severe damage lies at an average of 2 miles from the smelters, the boundary between severe and considerable damage at about 4 miles, and between considerable and moderate damage at about 4.5 miles. The outer limit for moderate damage seemed to lie at about 6 miles from the smelters. Thus it appears that the aerial survey agrees reasonably well with the local lakeside notes, when one considers that a good deal of overlap occurs in both sets of records.

TABLE III

Estimates of vegetation damage in relation to distance from the sources of aerial smelter pollution

	Miles from smelter stacks														
Damage	0.5	1.0	1.5	2	3	4	5	6	7	8	10	12	15	20	40
Very severe	1	3	3												
Severe	2	2	5	5	3	2									
Considerable		1	2	5	7	10			1						
Moderate					3	5				1					
Not obvious					1	1	2	4	5	2	10	3	8	4	

While close and expert examination of the flora might well have revealed injury from sulphur dioxide fumes at distances much greater than 5 miles from the smelters (cf. refs. 10, 11), the present records provide the kind of estimate that might be made by untrained observers, and so may be useful in judging the influence of smelter pollution upon the amenity value of the vegetation. A more detailed study of vegetation NNE. of Falconbridge (6) showed a generally comparable picture of damage, the numbers of macrophyte species declining sharply within about 4–7 miles of the smelter, and the proportion of open ground increasing in inverse ratio.

It may be remarked that no assessment is given for the effect of wind direction upon the distance to which pollution effects extend. Although one might expect the effects to be most marked in the NE. direction of the prevailing wind, variations in terrain, fire damage, and human interference render any such assessment extremely difficult, and it has not been attempted.

The effects of sulphur pollution upon vegetation may be examined in yet another way, by correlating the qualitative estimates of local damage with the sulphate concentrations in the nearby lake waters. This evident correlation is

demonstrated in Table IV. It may also be of interest to give the logarithmic averages of sulphate concentration for lakes in areas suffering different degrees of pollution damage. Where damage is very severe, sulphate averages nearly 3 meq/l., as against 1.2 meq in severe cases and 0.7 meq where damage is recorded as considerable. In moderately damaged sites, sulphate levels average 0.5 meq/l., while in sites showing no obvious damage the average concentrations are only about 0.4 meq/l.

TABLE IV

Lake water sulphate in relation to damage of surrounding vegetation by smelter pollution

Sulphate, meq/l.	Vegetation damage				
	Very severe	Severe	Considerable	Moderate	Not obvious
0.21–0.40		1	4	1	19
0.41–0.60		1	4	6	16
0.61–0.80		2	9	1	4
0.81–1.00		1	1		
1.01–1.25		5	3		
1.26–1.50	2	3	2		
1.51–2.00	1	2	1		
2.01–4.00	1	2	1		
>4.00	3				

Concluding Remarks

The present study indicates the extent of damage in the vicinity of the Sudbury group of smelters. However, its potential application in connection with the location of future smelters or similar industrial installations elsewhere may also be of interest. For example, should a new smelter be contemplated then its location should be sufficiently far from residential and other community areas so that their amenities may be preserved from pollution and damage. For smelters of capacity within the range of those at Sudbury, a distance of more than 5 miles should be recommended. In addition a preliminary survey might reveal situations in which pollution damage could be restricted as far as possible to the less productive land types, leaving those areas with a greater proportion of productive land types for forest or agricultural use.

Acknowledgments

We are much indebted to Mr. Keith Acheson, Regional Director of the Ontario Department of Lands and Forests at Sudbury, for arranging the use of a helicopter, and to Mr. R. F. Ross for piloting us to so many otherwise inaccessible locations.

References

1. Cole, R. J. 1949. The removal of sulphur gases from smelter fumes, 1947. A report by the Ontario Research Foundation. The King's Printer, Toronto.
2. Dreisinger, B. R. 1959. Interim report for 1958 to the Special Sulphur Dioxide Committee. Atmospheric sulphur dioxide measurements and the effects of the gas upon vegetation in the Sudbury area. Mimeographed. Dept. of Mines, Sudbury, Ontario.
3. Gorham, E. 1957. The chemical composition of lake waters in Halifax County, Nova Scotia. Limnology & Oceanog. **2**, 12–21.

4. GORHAM, E. 1958. Free acid in British soils. Nature, **181**, 106.
5. GORHAM, E. 1958. Free acid in Minnesota podzols. Ecology, **39**, 770.
6. GORHAM, E. and GORDON, A. G. 1960. Some effects of smelter pollution northeast of Falconbridge, Ontario. Can. J. Botany, **38**, 307–312.
7. HERON, J. and MACKERETH, F. J. H. 1954. The estimation of calcium and magnesium in natural waters, with particular reference to those of low alkalinity. Mitt. Intern. Ver. Limnol., No. 5.
8. LINZON, S. N. 1958. The influence of smelter fumes on the growth of white pine in the Sudbury region. Joint publication of Ont. Dept. of Lands and Forests and Ont. Dept. of Mines, Toronto.
9. MACKERETH, F. J. H. 1955. Ion-exchange procedures for the estimation of (I) total ionic concentration, (II) chlorides and (III) sulphates in natural waters. Mitt. Intern. Ver. Limnol., No. 4.
10. MCCALLUM, A. W. 1958. Report on field examination of the Sudbury area, July 11–20, 1957. Mimeographed. Dept. of Agriculture, Ottawa.
11. MCCALLUM, A. W. 1959. Report on field examination of the Sudbury area, July 11–18, 1958. Mimeographed. Dept. of Agriculture, Ottawa.

INDUCED SYNTHESIS OF PHLORIN-C^{14} IN PELARGONIUM[1]

CHITRA ROY, P. N. AVADHANI,[2] AND G. H. N. TOWERS

Abstract

Leaf disks of *Pelargonium* were shown to be capable of synthesizing phlorin-C^{14} when administered phloroglucinol together with C^{14}-labelled glucose, sucrose, or succinic acid. Changes with time in the distribution of radioactivity in phlorin and in sucrose in disks maintained on phloroglucinol and glucose-C^{14}, in the light or in the dark, were followed. Phlorin-C^{14} synthesis predominated in the dark and sucrose-C^{14} synthesis in the light. The steady accumulation of phlorin with time suggests that it is not utilized metabolically. Labelled phlorin and sucrose were formed when succinate-1,4-C^{14} or succinate-2,3-C^{14} was administered in the light. Labelled hexoses were obtained with succinate-2,3-C^{14} in the dark. Possible pathways for the conversion of succinate to sugars are suggested. In these experiments the synthesis of sucrose-C^{14} from succinate-C^{14} was reduced in the presence of phloroglucinol, concomitant with the formation of phlorin-C^{14}.

Introduction

When phenols, in low concentrations, are assimilated by plant tissues β-glucosides of these phenols are synthesized. This was first shown in 1916 by Ciamician and Ravenna (11) using maize and bean plants. An extensive study of this process was carried out by Miller (15, 16, 17, 18, 19, 20), who showed that compounds containing an alcoholic or a phenolic hydroxyl group, when introduced into plants, either in solution or as a vapor, combine with sugars within the plant to form β-glycosides. Miller showed that the sugar component of the glycosides thus formed varied with the species of the plant and with the aglycone furnished. Several reports regarding the synthesis of glucosides from administered phenols have appeared recently (14, 21, 22, 25, 28). The synthesis in vitro of phenolic glucosides has also been demonstrated (9, 10, 21).

When phloroglucinol is absorbed by plant tissues it is rapidly converted to the β-glucoside phlorin (14). The present report is concerned with the competition between phlorin synthesis and sucrose synthesis in *Pelargonium* when C^{14}-labelled glucose, sucrose, succinate-1,4-C^{14}, or succinate-2,3-C^{14} are fed together with phloroglucinol.

Materials and Methods

Radioactive Chemicals

Glucose-U-C^{14}, sucrose-U-C^{14}, and succinate-1,4-C^{14} were purchased from the Atomic Energy of Canada Ltd. Succinate-2,3-C^{14} was purchased from Research Specialities Inc., Richmond, California.

Administration of C^{14}-labelled Compounds

In each treatment, five leaf disks, from mature leaves of *Pelargonium hortorum* L. H. Bailey var. Carmine Pink, 1.2 cm in diameter obtained with a Ganong leaf punch, were infiltrated under vacuum with tap water, blotted, and placed in a Petri dish in a solution of the appropriate radioactive compound together with a 10 m*M* phloroglucinol solution where necessary. The

[1]Manuscript received March 30, 1960.
Contribution from the Department of Botany, McGill University, Montreal, Que.
[2]National Research Council Postdoctoral Fellow, 1958–60.

Petri dishes were covered and placed under a bank of fluorescent lights (approximately 200 ft-c) for light treatment or placed in a darkroom for dark treatment.

Extraction and Identification of Compounds

After incubation, the disks were washed in tap water, using a standard procedure, blotted, and killed by immersion in 75 ml of boiling 80% ethanol. The disks were exhaustively extracted by refluxing with two changes of ethanol. The combined ethanolic extracts were evaporated to dryness at room temperature by means of a jet of filtered air. The dried residue was redissolved in 1 ml of 80% ethanol and aliquots plated for radioactivity measurements. Separate aliquots were spotted on large sheets of Whatman No. 1 paper for chromatography. Two-directional chromatograms were made, the solvents consisting of water-saturated phenol for the first direction followed by the upper phase of a 4:1:5 mixture of butanol:acetic acid:water for the second direction.

Phlorin and phloroglucinol were detected by spraying chromatograms with diazotized *p*-nitroaniline (27), diazotized sulphanilic acid (3), or, most conveniently, with Ehrlich reagent (*p*-dimethylaminobenzaldehyde in ethanolic HCl) which gives a red color with these compounds. The first two reagents are not always satisfactory, particularly with freshly prepared chromatograms, due to the presence of phenol and its decomposition products on the paper which produce a highly colored background. Phlorin was identified by co-chromatography with phlorin obtained by alkali fission of phloridzin according to the method of Cremer and Seuffert (12). Hydrolysis of phlorin gave phloroglucinol and glucose which were also identified chromatographically. Sugars were identified with aniline oxalate or benzidine – trichloroacetic acid (3) sprays. Amino acids were detected with ninhydrin.

Measurement of Radioactivity

Radioactive spots on chromatograms were located by the preparation of radioautographs. Radioactivity was determined by direct counting on the paper using a thin end-window G.M. tube (Nucleonics type IWAAA) attached to a Berkeley Decimal scaler. Total activity in ethanolic extracts was determined by plating suitable aliquots on aluminum planchets and counting at infinite thinness using the same equipment.

Experimental and Results

Variability between Different Sets of Disks Given Identical Treatment

Each of four sets of five leaf disks was administered 2 ml phloroglucinol and 0.2 ml glucose-C^{14} (0.8 mM, 2 μc) in the light for 16 hours. Table I shows the variability between different sets in total ethanol-soluble activity and in the distribution of radiocarbon in the major compounds.

Synthesis of Phlorin-C^{14} and Sucrose-C^{14} in Light and Dark

Two sets of leaf disks were administered 0.5 ml phloroglucinol and 0.2 ml glucose-U-C^{14} (0.8 mM, 2 μc) for 24 hours, one set being kept in the light

TABLE I

Distribution of radioactivity in 80% ethanol-soluble fraction of sets of leaf disks administered phloroglucinol and glucose-C^{14}

Set No.	Radioactivity				
	Total (c.p.m.)	Per cent distribution			
		Phlorin	Glucose	Fructose	Sucrose
1	51,500	20	21	21	33
2	53,100	27	14	13	30
3	48,100	17	22	23	30
4	48,300	21	18	17	32

TABLE II

Distribution of radioactivity in 80% ethanol extracts of leaf disks administered phloroglucinol and glucose-C^{14} in light and in dark

Treatment	Radioactivity					
	Total (c.p.m.)	Per cent distribution				
		Phlorin	Glucose	Fructose	Sucrose	Others
Light	26,700	48	3	3	24	22
Dark	21,800	54	1	1	14	30

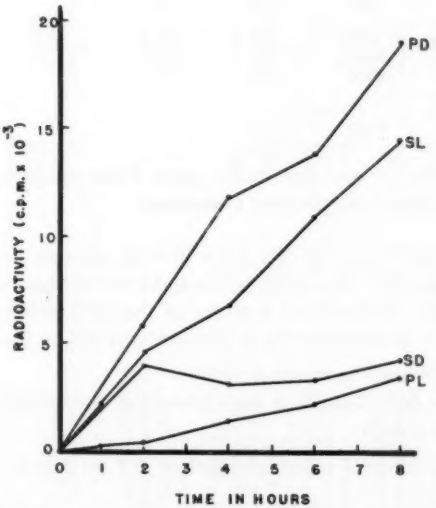


FIG. 1. Accumulation of radioactivity, with time, in phlorin and in sucrose in *Pelargonium* leaf disks administered glucose-U-C^{14} and phloroglucinol. PD—phlorin-C^{14}, leaf disks in the dark; PL—phlorin-C^{14}, leaf disks in the light; SD—sucrose-C^{14}, leaf disks in the dark; SL—sucrose-C^{14}, leaf disks in the light.

and the other in the dark. The results, shown in Table II, indicate that less sucrose was synthesized in the dark. This observation was confirmed by the following experiment.

Ten sets of disks were administered phloroglucinol and glucose-C^{14} as in the above experiment. Five of the sets were subjected to the light treatment and the other five to the dark treatment. Two sets, one from each treatment, were killed after 1, 2, 4, 6, and 8 hours from the start of the experiment. The results of this experiment are summarized in Fig. 1.

A third experiment was carried out in which five sets were administered phloroglucinol and glucose-C^{14} as before and subjected to the light treatment. Four of these sets, one at a time, were killed after 1, 2, 4, and 6 hours from the start of the experiment. After the fourth set was killed, the fifth set was transferred to the dark and was killed after a further 18-hour period. The results of this experiment are shown in Table III.

In all these experiments radioactive alanine was detected on chromatograms only in the case of dark treatments.

TABLE III

Changes with time in the distribution of C^{14} in the ethanol-soluble fraction of disks fed glucose-C^{14} and phloroglucinol

Treatment	Radioactivity					
	Total activity (c.p.m.)	Per cent distribution				
		Phlorin	Sucrose	Glucose	Fructose	Others
1 hour in light	630	18	45	11	1 (trace)	25
2 hours in light	870	23	38	3	4	32
4 hours in light	2400	28	32	3	7	30
6 hours in light	4000	38	27	3	3	29
6 hours in light followed by 18 hours in the dark	5300	36	3	5	4	52

Synthesis of Phlorin-C^{14} and Sucrose-C^{14} from Phloroglucinol Administered together with Different Radioactive Compounds

Sucrose-C^{14}

When sucrose-U-C^{14} (0.2 ml of a 3.5×10^{-4} *M* solution with an activity of 2 μc) was administered to leaf disks in the light for 16 hours in the presence of phloroglucinol, 42% of the total activity of the 80% ethanolic extracts was in phlorin-C^{14}, 32% in sucrose, 5% in glucose, and 8% in fructose.

Succinate-1,4-C^{14} and Succinate-2,3-C^{14}

Two sets of five leaf disks each were placed in the following solutions and left in the light for 1 hour:

Set 1: 0.5 ml of 10 m*M* phloroglucinol + 0.2 ml 3 m*M* succinate-1,4-C^{14} (2 μc);

Set 2: 0.5 ml tap water + 0.2 ml 3 m*M* succinate-1,4-C^{14} (2 μc).

In order to test the effect of light, a second series of similar experiments was carried out with four sets of 10 leaf disks each. These were maintained either in the light or in the dark for 5¾ hours in the following solutions:

TABLE IV

Effect of phloroglucinol on the distribution of radioactivity in leaf disks administered succinate-1,4-C^{14} or succinate-2,3-C^{14} in light and dark

Expt. No.	Compounds administered	Time, hours	No. of leaf disks	Experimental conditions	Radioactivity in 80% ethanol fraction				
					Total (c.p.m.)	Per cent distribution			
						Phlorin	Sucrose	Hexoses	Others
1	Succinate-1,4-C^{14} + phloroglucinol	1	5	Light	34,000	58	15	....27....	
	Succinate-1,4-C^{14} + water	1	5	Light	37,400	0	55	....45....	
2	Succinate-1,4-C^{14} + phloroglucinol	5¾	10	Light	151,500	37	9	6	44
	Succinate-1,4-C^{14} + water	5¾	10	Light	95,000	0	27	9	64
	Succinate-1,4-C^{14} + phloroglucinol	5¾	10	Dark	18,400	0	0	0	100
	Succinate-1,4-C^{14} + water	5¾	10	Dark	22,000	0	0	0	100
3	Succinate-2,3-C^{14} + phloroglucinol	5¾	10	Light	53,800	9	11	21	59
	Succinate-2,3-C^{14} + water	5¾	10	Light	60,000	0	46	19	35
	Succinate-2,3-C^{14} + phloroglucinol	5¾	10	Dark	11,300	0	0	12	88
	Succinate-2,3-C^{14} + water	5¾	10	Dark	16,400	0	0	25	75

Set 3: 1 ml of 10 m*M* phloroglucinol + 0.2 ml 3 m*M* succinate-1,4-C^{14} (2 μc);

Set 4: 1 ml of tap water + 0.2 ml 3 m*M* succinate-1,4-C^{14} (2 μc).

These two sets were maintained in the light. Sets 5 and 6 were identical with sets 3 and 4 and were maintained in the dark.

In another four sets of feedings (sets 7–10) similar to those of sets 3–6, the succinate-1,4-C^{14} was replaced by succinate-2,3-C^{14}. The activities, concentrations of solutions, volumes, etc. were kept as nearly as possible to those of sets 3–6.

The results of these 10 feedings are shown in Table IV.

Discussion

The results outlined in Table I show that there was a difference of approximately 6% in the total activities of ethanol extracts of different sets. Although there was a much wider variation in the distribution of radioactivity in the four major radioactive compounds, the ratio of activity between glucose-C^{14} and fructose-C^{14} was approximately 1:1. The results shown in Table II indicate that phlorin-C^{14} synthesis occurred both in light and in dark. This observation is in agreement with that of Nystrom *et al.* (22), who found that the synthesis of phenylglucoside was unaffected by light. However, it was also noted, in the same experiment (Table II), that the accumulation of activity in sucrose was much lower in the dark than in the light, suggesting that light energy is essential for the maintenance of high activity in sugars. This observation is in agreement with that of other workers (6, 7).

While Table II shows no difference in the accumulation of radioactivity after 24 hours whether the feeding was in the light or in the dark, the results of a subsequent experiment, shown in Fig. 1, indicate quite definitely that light effects sucrose synthesis and glucoside formation. The rate of incorporation of C^{14} into sucrose was much faster in the light than in the dark. This is in agreement with the results of the previous experiment. However, during this period, the rate of accumulation of activity into phlorin was much higher in the dark than in the light (Fig. 1). Furthermore, the amount of C^{14} incorporated into phlorin in the dark was comparable with that accumulated in sucrose in light. In other words, the combined activity of phlorin and sucrose was almost equal both in light and in the dark. Thus, a competition between sucrose synthesis and phlorin synthesis is clearly evident.

The lowered activity in sucrose-C^{14} of the disks transferred to the dark after 6 hours in light is shown in Table III. This table also shows that although the rate of sucrose-C^{14} synthesis in these disks was not as great as in the previous sets, the activity in sucrose at the end of the 24-hour period was considerably lower. The steady accumulation of phlorin with time suggests that it is not utilized metabolically in the disks.

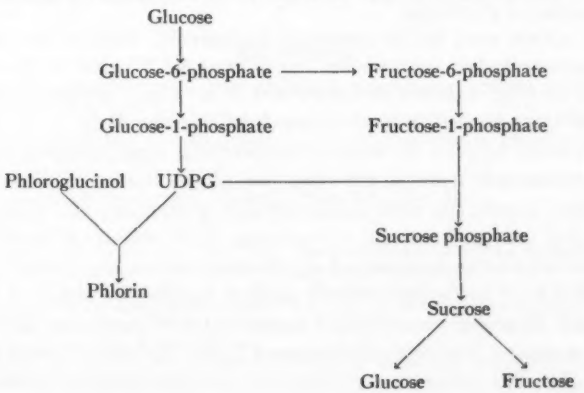
When sucrose-U-C^{14} was fed to the disks, both free glucose and fructose were found to be radioactive.

Table III shows that free fructose was labelled fairly early in these experiments. These results are contrary to the findings of Putman and Hassid (26) where they were unable to detect any free fructose in the leaves of *Canna indica* administered glucose-C^{14}. Porter and May (23) also state that no free fructose-C^{14} was detected in tobacco leaf disks administered glucose-C^{14}. Vittorio *et al.* (29), however, found that the specific activity of free fructose in tobacco leaf disks administered glucose-C^{14} was much less than that of the fructose moiety of sucrose-C^{14}. The early appearance of fructose in our experiments suggests either that sucrose is readily hydrolyzed in the tissue or that fructose is an intermediate in sucrose synthesis.

In spite of the fact that the present work is chiefly concerned with the induced synthesis of phlorin, the following discussion concerning the synthesis of sucrose appears relevant because of the observed competition between sucrose synthesis and glycoside formation. Two schemes have been suggested for the synthesis of sucrose, one involving the combination of uridinediphosphate glucose (UDPG) with fructose; the other involving the combination of UDPG with fructose-1-phosphate (5). Both these reactions have been shown to occur in vitro. The main objection to the operation of the first scheme in intact tissue stems from the work on photosynthesis (1) and the results obtained with the feeding of labelled hexoses (23, 26, 29), in which free fructose was not labelled before sucrose. Free fructose-C^{14} was considered to arise as a result of the hydrolysis of sucrose-C^{14}.

Cardini and Leloir (9) have shown that UDPG is an intermediate in the synthesis of arbutin, the glycoside of hydroquinone. Cardini and Yamaha (10) showed that other sugar derivatives or sugars, including sucrose, were not effective in glycoside synthesis. Pridham (24), however, has presented evidence to the effect that trans-glycosidation, with β-glucosidase, is involved in the synthesis of phenolic glucosides. In the present work obviously it is not possible to distinguish between the two.

A scheme, based on the studies of Cardini and Leloir (9), Burma and Mortimer (5), and the results presented in this paper, is outlined below.



In the photosynthesis studies of Burma and Mortimer (6) they have suggested that one of the factors involved in sucrose formation is the light-dependent production of phosphate bond energy. They have shown that there is an inverse relationship between the formation of sucrose and the formation of alanine. Bidwell *et al.* (2) also found an accumulation of alanine-C^{14} when glucose-C^{14} was fed to wheat leaves in the dark. Our results are in agreement with these observations.

The ready incorporation of C^{14} into phlorin and into fructose and glucose from sucrose-U-C^{14} (see Experimental) also supports the idea that the latter can be readily hydrolyzed. Putman and Hassid (26) are of the opinion that such a hydrolysis is extracellular. Our results show, however, that 8% of the activity from sucrose-C^{14} was in free fructose as compared with 5% in glucose. This and the fact that labelled fructose was readily formed from labelled glucose suggests that sucrose is hydrolyzed intracellularly and that the resulting glucose moiety is available for glycoside synthesis. Dormer and Street (13) have suggested that the enzymes concerned with the phosphorylation and dephosphorylation of sugars are present in the protoplast surface.

The results of the three experiments, where succinate labelled in different positions was administered separately and together with phloroglucinol under different conditions, are of considerable interest from the viewpoint of succinate metabolism in plants. Experiment 1 shows quite clearly that in light succinate-C^{14} can give rise to sucrose-C^{14} in *Pelargonium* leaf disks. In the presence of phloroglucinol it gave rise to phlorin-C^{14} at the expense of sucrose-C^{14}. In this respect, succinate-C^{14} was found to be as effective as glucose-C^{14} in light.

In the light of recent knowledge of carbohydrate and organic acid metabolism in plants the conversion of succinate-C^{14} to labelled sugars can conceivably proceed in a number of ways, of which the following two appear most likely:

(*a*) Succinate-1,4-C^{14} is decarboxylated to give $C^{14}O_2$ which is converted to sugars by photosynthetic fixation.

(*b*) The 3-carbon compound remaining after decarboxylation forms sugars by a reversal of glycolysis.

If (*a*) above were to be operating exclusively, little or no radioactivity would be expected in sugars when succinate-1,4-C^{14} is fed in the dark. That this may be true is shown in experiment 2, in which neither phlorin-C^{14} nor sucrose-C^{14} was formed from succinate-1,4-C^{14} in the dark.

On the other hand, if (*b*) were to be operating, some labelling in the sugars would be expected even in the dark, i.e., in the absence of photosynthetic assimilatory power. In view of the absence of such activity it is possible to suggest that only pathway (*a*) is operating. If, however, (*a*) is the only pathway involved in the synthesis of sugars from succinate, it follows that with succinate-2,3-C^{14} the sugars cannot become radioactive either in the light or in the dark. However, experiment 3 shows that with succinate-2,3-C^{14}, phlorin-C^{14} and sucrose-C^{14} as well as fructose-C^{14} and glucose-C^{14} were synthesized in the light. The latter two compounds were synthesized in the dark also.

Thus, the results of experiment 3 suggest the operation of pathway (*b*). The failure to synthesize phlorin-C^{14} or sucrose-C^{14} with succinate-2,3-C^{14} in the dark is not understood.

To explain the lack of activity in sugars in the dark in experiment 2, it would be necessary to suggest that the 3-carbon compound is further decarboxylated to yield acetate. This acetate could then form the sugars. In experiments 1 and 2 with succinate-1,4-C^{14}, this acetate would be non-radioactive. Hence in these experiments sugars are labelled only in photosynthesis (refixation), whereas with succinate-2,3-C^{14} the acetate would be radioactive as also would be the sugars derived therefrom. This was found to be true in experiment 3. Conversion of acetate to sugars has been demonstrated by a number of workers (see ref. 4).

The evidence here suggests that when succinate-1,4-C^{14} was fed, the *radioactive* sugars were formed essentially via the first scheme outlined above but that when succinate-2,3-C^{14} was fed, they were synthesized by a second route. In other words, two pathways are involved in the synthesis of sugars from succinate and this is supported by the following observation.

Pathway (*a*), which leads to the formation of labelled sugars from succinate-1,4-C^{14}, was not inhibited by phloroglucinol as shown by the combined activities in sugars from experiment 2, whereas the formation of labelled sugars by the second pathway from succinate-2,3-C^{14} was inhibited by phloroglucinol.

The succinate-C^{14} feeding experiments were designed to show the competition between sucrose synthesis and phlorin synthesis not only from glucose-C^{14} but also from another carbon compound which we know to be capable of forming sugars in *Pelargonium* leaves. It was not intended to be a study of the transformation of succinate to sucrose. Further studies on the relatively rapid conversion of exogenous succinic acid to sugars are contemplated.

Acknowledgments

We wish to thank the National Research Council of Canada for the award of a studentship to one of us (C.R.) and for financial support of this work.

References

1. Benson, A. A. and Calvin, M. 1950. Carbon dioxide fixation by green plants. Ann. Rev. Plant Physiol. **1**, 25–42.
2. Bidwell, R. G. S., Krotkov, G., and Reed, G. B. 1955. The influence of light and dark on the metabolism of radioactive glucose and glutamine in wheat leaves. Can. J. Botany, **33**, 189–196.
3. Block, R. J., Durrum, E. L., and Zweig, G. 1955. A manual of paper chromatography and paper electrophoresis. Academic Press, Inc., New York.
4. Bradbeer, C. and Stumpf, P. K. 1959. Fat metabolism in higher plants. Ann. Rev. Plant Physiol. **10**, 197–222.
5. Burma, D. P. and Mortimer, D. C. 1956. The biosynthesis of uridine diphosphate glucose and sucrose in sugar beet leaf. Arch. Biochem. Biophys. **62**, 16–28.
6. Burma, D. P. and Mortimer, D. C. 1957. The fate of assimilated $C^{14}O_2$ in the sugar beet leaf studied by displacement with $C^{12}O_2$. Can. J. Biochem. and Physiol. **35**, 835–843.
7. Calvin, M. and Massini, P. 1952. The path of carbon in photosynthesis. XX. The steady state. Experentia, **8**, 445–457.
8. Cardini, C. E., Leloir, L. F., and Chiriboga, J. 1955. The biosynthesis of sucrose. J. Biol. Chem. **214**, 149–155.

9. CARDINI, C. E. and LELOIR, L. F. 1957. Biosintesis de glucosidos. Ciencia e invest. Buenos Aires, **13**, 514.
10. CARDINI, C. E. and YAMAHA, T. Biosynthesis of plant glycosides from uridine diphosphate glucose. Nature, **182**, 1446–1447.
11. CIAMICIAN, G. and RAVENNA, C. 1916. Atti reale accad. Lincei, **25**, 3. (Quoted from: Armstrong, E. F. and Armstrong, K. F. 1931. The glycosides. Longmans, Green & Co., Ltd., London.)
12. CREMER, M. and SEUFFERT, R. W. 1912. Uber Phlorin, ein Spaltungsprodukt des Phloridzin. Ber. **45**, 2565–2571.
13. DORMER, K. J. and STREET, H. E. 1949. The carbohydrate nutrition of tomato roots. Ann. Botany London, n.s. **13**, 199–217.
14. HUTCHINSON, A., ROY, C., and TOWERS, G. H. N. 1958. Synthesis of phlorin and other phenolic glucosides by plant tissues. Nature, **181**, 841–842.
15. MILLER, L. P. 1938. Formation of β-(2-chloroethyl)-d-glucoside by gladiolus corms from absorbed ethylene chlorohydrin. Contribs. Boyce Thompson Inst. **9**, 425–429.
16. MILLER, L. P. 1939. Synthesis of β-(2-chloroethyl)-d-glucoside by potato tubers treated with ethylene chlorohydrin. Contribs. Boyce Thompson Inst. **10**, 139–141.
17. MILLER, L. P. 1939. Glycoside formation in plants from absorbed ethylene chlorohydrin, *o*-chlorophenol and chloral hydrate. Am. J. Botany, **26**, 14s.
18. MILLER, L. P. 1940. Formation of β-*o*-chlorophenyl-gentiobioside in gladiolus corms from absorbed *o*-chlorophenol. Contribs. Boyce Thompson Inst. **11**, 271–279.
19. MILLER, L. P. 1941. Simultaneous formation of β-gentiobioside and a β-glucoside in gladiolus corms treated with chemicals. Contribs. Boyce Thompson Inst. **12**, 163–166.
20. MILLER, L. P. 1957. Monosaccharide components of an induced glycoside of trichloroethyl alcohol from dandelion tops. Contribs. Boyce Thompson Inst. **19**, 113–115.
21. MIWA, T., NAKAMURA, S., and SHIBATA, A. 1958. Biosynthesis of arylglycoside in higher plants. Koso Kaguku Shinpojiumu, **12**, 48–53 (from Chem. Abstr. **32**, 1314).
22. NYSTROM, C. W., TOLBERT, N. E., and WENDER, S. H. 1959. Determination of β-phenylglucoside in plant leaves. Plant Physiol. **34**, 142–143.
23. PORTER, H. K. and MAY, L. H. 1955. Metabolism of radioactive sugars by tobacco leaf discs. J. Exptl. Botany, **6**, 43–63.
24. PRIDHAM, J. B. 1957. Determination of phenolic glycosides and aglycones on paper chromatograms. Anal. Chem. **29**, 1167–1169.
25. PRIDHAM, J. B. 1958. Metabolism of phenolic compounds by the broad bean *Vicia faba*. Nature, **182**, 795–796.
26. PUTMAN, E. W. and HASSID, W. Z. 1954. Sugar transformation in leaves of *Canna indica*. I. Synthesis and inversion of sucrose. J. Biol. Chem. **207**, 885–902.
27. SWAIN, T. 1953. The identification of coumarins and related compounds by filterpaper chromatography. Biochem. J. **53**, 200–208.
28. TOWERS, G. H. N., HUTCHINSON, A., and ANDREAE, W. A. 1958. Formation of a glycoside of maleic hydrazide in plants. Nature, **181**, 1535–1536.
29. VITTORIO, P. V., KROTKOV, G., and REED, G. B. 1954. Synthesis of radioactive sucrose by tobacco leaves from C^{14} uniformly labelled glucose and glucose-1-phosphate. Can. J. Botany, **32**, 369–377.

DISTRIBUTION OF CANAVANINE IN THE FAMILY LEGUMINOSAE AS RELATED TO PHYLETIC GROUPINGS[1]

B. A. Birdsong, R. Alston, and B. L. Turner

Abstract

The seeds of 161 species of legumes, representing 78 genera, were examined by chromatographic methods for canavanine content. The results are supplemented by data, variously collected, by other authors, to yield a total of 219 investigated species representing 109 genera. Of these, 68 species belonging to 32 genera have canavanine in their seeds.

Canavanine has not been found in the subfamilies Mimosoideae or Caesalpinioideae. In the subfamily Papilionoideae it has not been found in the tribes Podalyrieae and Sophoreae. At least one species from each of the other tribes produces canavanine. In three genera, some species produce canavanine while others do not.

These data are presumed to be of taxonomic significance and have been interpreted as support for chromosomal information indicating that the tribes Mimosoideae and Caesalpinioideae diverged relatively early from the "stem" line which is now represented by the Papilionoideae.

Introduction

The amino acid canavanine is known to occur in the free state only in species of the family Leguminosae. Canavanine is commonly associated with the seeds of those species wherein it is found, but Tschiersch (1959) has reported the synthesis of canavanine in rooted leaves of *Canavalia* sp. Since its presence in plants can be detected by rather simple chromatographic techniques, it appears to be a suitable choice for correlative phytochemical–systematic study.

The weight of evidence indicates that canavanine is an important metabolite in those seeds in which it occurs. It is sometimes present in high concentration in seeds, and circumstantial evidence points strongly to its role in the transport as well as storage of nitrogen (Tschiersch 1959). It has been suggested that canavanine is a natural substrate of the enzyme arginase, which is known to occur in those legumes containing free canavanine (Bell 1958). If it is an important metabolite, as suspected, then its distribution is likely to be more vigorously controlled by selection pressure than, for example, a secondary substance with little selective value in itself, e.g. a pelargonidin versus cyanidin configuration among anthocyanins. Its distribution, accordingly, should be of greater significance. The present study was undertaken, therefore, with the expectation that the pattern of distribution of canavanine would prove to be of systematic significance provided that an adequate number of species were examined.

[1]Manuscript received February 22, 1960.

Contribution from the Plant Research Institute, The University of Texas, Austin, Texas; and the Department of Botany, University of Rhode Island, Kingston, Rhode Island. Supported in part from funds provided by the University Research Institute of the University of Texas.

Methods and Materials

Canavanine was extracted from dormant seeds and identified by a method essentially that of Bell (1958) which makes use of paper chromatography and the spraying reagent trisodium pentacyanoammonioferrate (PCAF). PCAF gives a distinctive magenta color only with canavanine and deaminocanavanine. The latter compound runs at an R_f distinctly higher than that of canavanine in the solvent systems used, and there was no evidence of its occurrence in the present study. No attempt was made to identify other substances which were disclosed by the PCAF spray although certain substituted guanidines react with PCAF to give colored products.

A few minor changes in the technique were introduced; e.g., the extracts were often allowed to stand overnight before chromatographing; the extracts were applied as ±4-cm streaks on the paper; and PCAF at a concentration of 0.25% (w/v) was found to be satisfactory. In early tests both the phenol and the butanol–pyridine solvents were used. Later, the butanol–pyridine solvent was used alone because its greater stability allowed a number of chromatograms to be run in succession in the same solvent.

Seeds for the present study were obtained from many sources, including packet material from various botanical gardens. Seeds of most of the tropical species were obtained from various correspondents and particular thanks are due to Dr. J. Moomaw, University of Hawaii; Dr. K. Chambers, Yale University; and Dr. A. Burkart, Instituto de Botánico Darwinion, Argentina, for making available seed materials from Africa, Central and South America respectively. Since this study is essentially a broad preliminary survey, no attempt has been made to voucher the species examined; however, the identifications are certainly accurate as to genera, though some of the species names applied to botanical garden packet material may be in error.

The arrangement of tribes and subtribes for the species listed in Table I follows the system of Taubert (1894).

TABLE I

Species of Leguminosae examined for canavanine

I. Subfamily Mimosoideae		II. Subfamily Caesalpinioideae	
1. Ingeae		1. Dimorphandreae	
**Enterolobium saman*	–	**Mora megistosperma*	–
2. Acacieae		2. Cynometreae	
Acacia dealbata	–	**Prioria copaifera*	–
Acacia farnesiana	–	3. Amherstieae	
3. Eumimoseae		**Afzelia quanzensis*	–
**Desmanthus cooleyi*	–	**Browneopsis* sp.	–
4. Adenanthereae		**Hymenaea courbaril*	–
**Adenanthera pavonia*	–	**Paramacrolobium* sp.	–
**Neptunia monosperma*	–	**Saraca cauliflora*	–
**Neptunia pubescens*	–	**Schotia* sp.	–
5. Piptadenieae		**Tamarindus indica*	–
**Entada leptostachya*	–	4. Bauhinieae	
6. Parkieae		*Bauhinia* (3 spp.)	–
**Parkia javanica*	–	**Bauhinia carronii*	–
**Parkia roxburgii*	–	**Bauhinia galpinii*	–
		**Cercis chinensis*	–
		**Cercis occidentalis*	–

TABLE I (*Continued*)

Species of Leguminosae examined for canavanine (*Continued*)

5. Cassieae	
Cassia (4 spp.)	−
**Ceratonia siliqua*	−
6. Eucaesalpinieae	
Caesalpinia pulcherrima	−
**Delonix regia*	−
**Gymnocladus dioca*	−
7. Sclerolobieae	
8. Swartzieae	
**Swartzia madagascariense*	−
**Swartzia* sp.	−
III. PAPILIONOIDEAE	
1. Sophoreae	
**Bolusanthus speciosus*	−
Calpurnia aurea	−
**Sophora tomentosa*	−
**Virgilea* sp.	−
2. Podalyrieae	
**Anagyris foetida*	−
Baptisia australis	−
**Daviesia corymbosa*	−
Piptanthus sp.	−
Thermopsis sp.	−
**Thermopsis macrophylla*	−
3. Genisteae	
3*a*. Lipariinae	
3*b*. Bossiaeinae	
**Bossiaea foliosa*	+
3*c*. Crotalariinae	
**Crotalaria erecta*	−
**Crotalaria lupulina*	−
**Crotalaria mucronata*	−
**Crotalaria mesopontica*	−
**Crotalaria virgulata*	−
3*d*. Spartiinae	
**Adenocarpus foliosus*	−
**Adenocarpus viscosus*	−
**Calycotome spinosa*	−
Laburnum (2 spp.)	−
Lupinus (2 spp.)	−
**Lupinus subcarnosus*	−
**Lupinus texensis*	−
**Spartium juncea*	−
3*e*. Cytisinae	
Cytisus sp.	−
**Cytisus proliferus*	−
**Sarothamnus scoparius*	−
4. Trifolieae	
Medicago (3 spp.)	+
**Medicago tuberculata*	+
**Medicago turbinata*	+
**Melilotus alba*	+
Melilotus (2 spp.)	+
Ononis sp.	+
**Trifolium bejariense*	+
**Trigonella caerulea*	+
**Trigonella foenum-graecum*	+
5. Loteae	
Anthyllis	+
**Dorycnium rectum*	+
**Dorycnium pentaphyllum*	+
**Hymenocarpus circinnatus*	+
Lotus corniculatus	+
**Lotus hispidus*	+
**Lotus oroboides*	+
Tetragonolobus palaestrinus	+
6. Galegeae	
6*a*. Indigoferinae	
Indigofera sp.	+
**Indigofera hirsuta*	+
**Indigofera texana*	+
**Indigofera tinctoria*	+
6*b*. Psoraliinae	
Amorpha fragrans	−
Amorpha fruticosa	−
**Petalostemum candidum*	−
**Petalostemum pulcherrimum*	−
**Psoralea cinerea*	−
**Psoralea floribunda*	−
**Psoralea graveolens*	−
**Psoralea patens*	−
6*c*. Brongniartinae	−
**Harpalyce arborescens*	−
6*d*. Tephrosiinae	
**Mundulea sericea*	−
**Tephrosia candida*	−
**Tephrosia ehrenbergiana*	−
**Tephrosia mesopontica*	−
**Wistaria sinensis*	+
6*e*. Robiniinae	
**Coursetia axillaris*	+
**Gliricidia sepium*	+
**Olneya tesota*	+
Robinia hispida (2 spp.)	+
**Sesbania* (6 spp.)	
6*f*. Coluteinae	
**Clianthus formosus*	+
Colutea arborescens	+
**Swainsonia colutoides*	−
Swainsonia hybrida	−
6*g*. Astragalinae	
Astragalus (2 spp.)	+
**Astragalus* sp.	+
**Astragalus wootonii*	−
Caragana arborescens (5 spp.)	+
**Glycyrrhiza glabra*	+
7. Hedysareae	
7*a*. Coronillinae	
Coronilla (2 spp.)	+
**Hippocrepis comosa*	−
**Hippocrepis unisiliquosa*	−
Ornithopus sp.	+

TABLE I (*Concluded*)

Species of Leguminosae examined for canavanine (*Concluded*)

7*b*. Euhedysarinae	
Hedysarum sp.	+
**Hedysarum alpinum*	+
**Hedysarum cinerascens*	+
**Hedysarum coronarium*	+
**Hedysarum sibiricum*	+
7*c*. Aeschynomeninae	
**Aeschynomene americana*	−
**Chaetocalyx* sp.	−
**Cyclocarpa* sp.	−
**Nissolia* sp.	−
7*d*. Patagoniinae	
**Adesmia bicolor*	−
**Adesmia incana*	−
**Adesmia latifolia*	−
7*e*. Stylosanthinae	
**Stylosanthes biflora*	−
**Zornia wundanyi*	−
7*f*. Desmodiinae	
Desmodium canadense	+
Desmodium gyrans	+
**Lespedeza cryptobotrya*	−
**Lespedeza cuneata*	−
**Lespedeza hedysaroides*	−
**Lespedeza inschanica*	−
**Lespedeza intermedia*	−
**Lespedeza juncea*	−
**Lespedeza procumbens*	−
**Lespedeza tomentosa*	−
**Lespedeza virginica*	−
**Uraria picta*	+
8. Dalbergieae	
8*a*. Pterocarpinae	
**Dalbergia ecastaphyllum*	−
**Dalbergia lanceolaria*	−
**Pterocarpus cambodianus*	−
8*b*. Lonchocarpinae	
**Lonchocarpus* sp.	+
**Lonchocarpus* sp.	+
8*c*. Geoffraeinae	
8*d*. Anomalae	
9. Vicieae	
**Abrus* sp.	−
**Cicer arietinum*	−
**Lathyrus hirsutus*	−
Lathyrus (2 spp.)	−
**Lens culinaris*	−
Vicia dasycarpa	+
**Vicia faba*	+
**Vicia ludoviciana*	+
**Vicia monantha*	+
Vicia sativa	+
**Vicia grandiflora*	−
**Vicia lathyroides*	−
**Vicia pannonica*	−
**Vicia pisiformis*	−
10. Phaseoleae	
10*a*. Glycineae	
Centrosema pubescens	+
**Centrosema virginiana*	+
**Cologania* (3 spp.)	−
**Glycine sinensis*	+
**Glycine soja* (7 "varieties" tested)	−
**Kennedya carinata*	+
**Kennedya prostrata*	+
**Kennedya stirlingii*	+
10*b*. Erythrineae	
Erythrina (2 spp.)	−
**Erythrina herbacea*	−
**Mucuna* sp.	+
**Stizolobium hassjoi* sp.	−
10*c*. Galactineae	
**Galactia canescens*	+
10*d*. Diocleinae	
Canavalia ensiformis	+
**Canavalia gladiata*	+
Canavalia lineata	+
Canavalia maritima	+
10*e*. Cajaninae	
**Cajanus indica*	−
**Eriosema* sp.	−
**Rhynchosia caribaea*	−
**Rhynchosia coryfolia*	−
**Rhynchosia minima*	−
10*f*. Phaseolinae	
**Dolichus bicontortus*	−
**Dolichus lablab*	−
**Dolichus leucomelis*	−
**Dolichus ornatus*	−
**Dolichus sesquipetalis*	−
**Phaseolus aborgineus*	−
**Phaseolus bracteatus*	−
**Phaseolus multiflorus*	−
**Phaseolus wrightii*	−
**Strophostyles helvola*	−
**Vigna cylindrica*	−
**Vigna repens*	−
**Vigna sesquipedalis*	−
**Vigna sinensis*	−

NOTE: * = first reported here, + = present, − = absent.

Results

Before the present study was undertaken, 58 species of legumes in 32 genera had been examined for canavanine. Of these, only 30 species in 16 genera were found to be positive for canavanine by the tests used here (Tschiersch 1959; Bell 1958).

We have investigated an additional 161 species belonging to 78 genera. These were selected to cover the major tribal and subtribal lines of the family Leguminosae. Of these previously uninvestigated taxa, 49 species belonging to 16 genera were found to be positive for canavanine.

To date, including all of the above, 219 species belonging to 109 genera have been examined by chromatographic means for canavanine; 68 species belonging to 32 genera are now known to give positive tests for this substance by the methods used (Table I).

Canavanine has not been found in the subfamilies Mimosoideae and Caesalpinioideae. In the subfamily Papilionoideae it has not been found in the tribes Podalyrieae and Sophoreae. It has been found in at least some of the species of all of the remaining tribes of Papilionoideae; however it is apparently rare in the tribe Genisteae, having been found in only one species (*Bossiaea foliosa*) of the 19 species in 9 genera investigated.

Canavanine is particularly abundant in the tribes Trifolieae and Loteae. All of the 12 species and 5 genera investigated for the former were found to give positive tests and this is true for all of the species investigated in the Loteae (7 species belonging to 5 genera).

The tribes Galegeae, Hedysareae, Dalbergieae, Vicieae, and Phaseoleae are quite variable with respect to species containing canavanine, but this presence or absence is often confined to species of a given subtribe (e.g. its complete absence in the 8 species of the subtribe Psoraliinae investigated, and 14 species of the subtribe Phaseolinae; and its presence in all of the 11 species and 6 genera of the subtribe Robininae, etc.).

Three genera (*Astragalus*, *Glycine*, and *Vicia*) were found to have species which tested negative for canavanine as well as species which tested positive (Table I).

Discussion

The taxonomic significance of any given character is perhaps best ascertained by the constancy of that character within any one taxon and its exclusion from any other related taxon which might be under consideration. Though most amino acids are widely distributed in nearly all plant groups, it is a singular fact that canavanine, so far as known, has been found in the free state only in the family Leguminosae (Bell 1958). Unlike such diverse biochemical substances as caffeine, nicotine, etc., free canavanine (and the enzyme system connected with its metabolism in the plant) appears to have arisen only in the family Leguminosae, and even more specifically, in the morphologically "advanced" subfamily Papilionoideae.

Tschiersch (1959) was of the opinion that since canavanine was found to be present in some species and absent in others of the same genus, its distribution in the Leguminosae had little, if any, taxonomic significance. However, the value of any given character in taxonomy is dependent upon the variation of that character within the group considered. For example, inferior versus superior ovaries is an important key character for the recognition of certain natural taxa, but in other equally natural groups, this character breaks down completely (e.g. Saxifragaceae). This in no way destroys the usefulness of this character where it is constant. Similarly, the presence or absence of canavanine in most of the tribes and/or subtribes listed in Table I appears to be significant. The sample has been broad enough to allow one to state with some confidence that canavanine probably does not occur in the free state in the subfamilies Mimosoideae and Caesalpinioideae, and furthermore, it suggests that species with the proper genomes for the formation of canavanine did not arise until the Papilionoid line was well established.

In spite of the limited sample from the tribes Sophoreae and Podalyrieae, it is tempting to offer the hypothesis that appropriate genomes for canavanine formation never developed in this line, this being consistent with the chromosomal information which indicates that these tribes are phyletic offshoots from the principal stock which gave rise to the remaining tribes in the subfamily Papilionoideae (Turner and Fearing 1959).

It is not surprising that certain large and diverse genera, such as *Vicia*, *Astragalus*, and *Glycine*, contain species with canavanine as well as some without it. Complex synthesis requiring several precursors will not be completed if any critical enzyme is missing from the sequence. Where canavanine has been found within a single natural genus, it is possible that the latent genomic potential for the production of certain precursors of canavanine perhaps exists imperfectly in other species in the group. Genetic recombination among such taxa with nonhomologous deficiencies would allow the appearance of canavanine. The principle is the same as that used in the testing of auxotrophic mutants of microorganisms such as *Neurospora* for complementary action. It may be possible to develop in canavanine-deficient genera genetic lines which store free canavanine in their seeds by appropriate crosses within such genera. Even under field conditions it appears that unexpected recombinations of this type occur. Turner and Alston (1959) found a substance in certain individuals from natural hybrid swarms of *Baptisia viridis* × *B. laevicaulis* but absent from individuals of "pure" populations of either parental type. However, what was apparently the same substance was found in another species, *B. leucantha*, taxonomically removed from both the parental species. The "new" compound found in individuals from the hybrid swarm is probably best explained by the fortuitous recombination of appropriate genes.

The distribution of canavanine in the Leguminosae, like chromosome numbers and exomorphic features, is meaningful in a phyletic system only when patterns are discernible. As indicated in Table I, there appears to be a significant total pattern within the family as well as within most taxa below the tribal

level. How important these data will be to any truly phyletic classification must await the evidence available from all fields, including the data from more inclusive biochemical studies. It is believed that the correlation of "biochemical profiles" (a term proposed by Alston and Turner (1959)) with data from morphological studies and cytogenetics will make for a closer approach to any truly phyletic classification.

References

ALSTON, R. and TURNER, B. L. 1959. Applications of paper chromatography to systematics: Recombination of parental biochemical components in a *Baptisia* hybrid population. Nature, **184**, 285–286.

BELL, E. A. 1958. Canavanine and related compounds in Leguminosae. Biochem J. **70**, 617–619.

TAUBERT, P. 1894. Leguminosae. *In* Engler and Prantl, Die natürlichen Pflanzenfamilien. **3** (3), 70–388.

TSCHIERSCH, B. 1959. Über canavanin. Flora, **147**, 405–416.

TURNER, B. L. and ALSTON, R. 1959. Segregation and recombination of chemical constituents in a hybrid swarm of *Baptisia laevicaulis* × *B. viridis* and their taxonomic implications. Am. J. Botany, **46**, 678–686.

TURNER, B. L. and FEARING, O. S. 1959. Chromosome numbers in the Leguminosae. II: African species, including phyletic interpretations. Am. J. Botany, **46**, 49–57.

OVULE DEVELOPMENT IN DIPLOID RED CLOVER[1]

BRONIUS POVILAITIS[2] AND J. W. BOYES[3]

Abstract

In red clover only one ovule of the two in each ovary regularly develops into a seed. Results of two experiments designed to reveal the fate of the ovules initially present provide a basis for comparing some features of ovule development in high- and low-seed-yielding plants. High-yielding plants produced embryo sacs in over 70% of ovules whereas low-yielding ones produced them in about 40%. The ability to produce embryo sacs seems to be genetically determined but strongly influenced by environment and occasionally by meiotic irregularities. Development started in about 85% of the fertile ovules in the high-yielding plants but in only about 60% of them in low-yielding plants. Apparently there was slow or limited growth of pollen tubes in the latter plants. Nearly 55% of the ovules developing just after fertilization matured into seeds in the high-yielding plants but only about 25% in the low ones. The higher frequency of ovule collapse in low-yielding plants seems to be a consequence of the delayed fertilization followed by retarded growth of the embryos and endosperms.

Although a few more ovules did start to develop in out-crosses than in sib-crosses, the more vigorous growth of the endosperm seems quite important in accounting for the superior seed yields in the out-crosses. Also a few more ovules started to develop when the pollen came from a high-yielding plant instead of a low-yielding one and this initial advantage seems to lead to improved seed production.

It is suggested that low-seed-producing plants may have certain genes that act like weak self-incompatibility alleles by inhibiting pollen tube growth, or, that their genetic constitutions are such that they cannot produce enough embryo sacs and/or support the growth of pollen tubes and ovules.

Introduction and General Observations

An ovary of red clover (*Trifolium pratense* L.) regularly contains two ovules but commonly only one ovule per ovary develops into a seed. In high-seed-yielding plants both ovules of each ovary usually produce mature embryo sacs and thus both ovules are available for fertilization and development (13). In low-seed-yielding plants one ovule per ovary often fails to produce a mature embryo sac and both fail in some ovaries. It is not surprising, therefore, that a relationship was found between the extent of production of mature embryo sacs and the seed yields of red clover plants. It is our intention to discuss in this paper the action of this and other factors that operate to reduce seed production.

Martin (8) described the process of embryo sac development in red clover and recently Pandey (9) has confirmed his findings in general. Martin and Pandey have also described the general course of embryo and endosperm development which we summarize briefly in the following statement for the convenience of the reader. In the red clover plants studied by the authors the fertilized eggs began to divide later than the primary endosperm nuclei. Late in June, 1954, the embryos had, on the average, 3, 45, and 600 cells at 24, 48, and 72 hours after pollination, respectively, and at the same respective

[1]Manuscript received December 17, 1959.
Contribution from the Departments of Genetics and Agronomy, Macdonald College, Que.
[2]Present address: Tobacco Substation, Dehli, Ontario.
[3]Department of Genetics, McGill University, Montreal, Que.

times 12, 120, and 900 nuclei were found in the endosperms. Under less favorable conditions of September, 1955, four cells per embryo were found at 48 hours, and 20 cells at 96 hours after pollination. The micropylar cells of proembryos divide more slowly and later develop into a large suspensor which under favorable conditions can be seen occasionally at 2 days after pollination. At the stage when cotyledons become noticeable, as in Fig. 7, the endosperm is already cellular except in the chalazal end of the nucellar cavity where it stays free nucleate. When the embryo is nearly mature, the endosperm is almost completely absorbed and only remnants of it can be found (Fig. 6).

At 2 and 4 days after pollination, one or more pollen tubes were often present, in the basal part of the style, in the ovary cavity, and in the micropyles of ovaries of the pollinated pistils. In several instances the pollen tube or tubes passed the entrance to the micropyle without entering in spite of the presence of an apparently normal embryo sac in the ovule (as also observed by Cooper and Brink (3) in alfalfa). In several cases ovules contained several-celled embryos together with an enlarged polar fusion nucleus (apparently unfertilized), and in several other cases an enlarged (apparently unfertilized) egg cell together with 32 or 64 endosperm nuclei. In general, however, pollen tube growth and fertilization proceeded normally.

In alfalfa, Cooper, Brink, and Albrecht (4) found that the average number of ovules per flower produced by both high- and low-seed-setting groups of plants was 9.3 and that in the high-seed-setting group an average of 3.1 ovules per flower became fertile, whereas in the low-seed-setting group only 2.1 became fertile. They also found 1.25 seeds per flower at maturity in the former group and only 0.07 in the latter. They, and a number of other workers, have described the collapse of fertilized ovules in legumes; e.g. Das (5) and Greenshields (6) in *Melilotus*, Martin (8) in *Trifolium pratense*, and Ledingham (7) in *Medicago sativa* and *M. falcata* crosses.

There are still many unsolved problems in fertilization and postfertilization development in legumes, and particularly in red clover. We hope that the results of two separate experiments, reported herein, will contribute some assistance in the solution of these problems.

Experiment I. Early Development in Relation to Seed Set following the Sibbing and Outcrossing of High- and Low-Seed-Yielding Plants

1. Materials and Methods

Two plants, belonging to separate lines of Dollard red clover growing in the field at Macdonald College in 1950, were allowed to produce seed under open pollination. The resulting progenies, *X* and *Y*, were grown in the field in 1952 and crossed to produce, among others, the 12 plants used in this experiment. Two sibs of progeny *X* were crossed to produce plants 1, 2, 8, and 9 of family 1 (see Table I); two sibs of progeny *Y* were crossed to produce plants 3, 6, 7, and 12 of family 2; two other sibs of progeny *Y* were crossed to produce plants 4 and 11 of family 3; and another sib of progeny *X* was crossed to another

sib of progeny *Y* to produce plants 5 and 10 of family 4. All 12 plants, plus their sibs, were grown in the field in 1953/54 and, according to seed yield under open pollination in 1953, plants 1–6 inclusive were rated as high-seed-yielding (H) and plants 7–12 as low-seed-yielding (L). Actual data on their seed yields (1953) and the percentages of fertile ovaries that they produced (1954) can be seen in Table 2 of our previous report (13).

In each family we made a comparable series of crosses, each series consisting of eight crosses (as for example, Table I, crosses 11–18). For each series we selected a low-yielding plant (as L-1) and a high-yielding one (as H-9), each having at least 12 heads with mature but unopened flowers. The heads were covered with cellophane bags for 2 days, then the bags were removed and the heads trimmed to 30–50 florets, and after completion of the pollinations as explained below, the heads were covered again with new bags. A set of three heads on each of the two selected plants received pollen from a low-yielding sib (say L-20); another set of three heads on each of the two plants received pollen from a high-yielding sib (say H-31); a third set of three heads on each of the two plants received pollen from a low-yielding non-sib plant (say L-88) of one of the other three families; and a fourth set of three heads on each of the two plants received pollen from a high-yielding non-sib plant (say H-98) of one of the other three families. Using S to indicate a sib-cross and C to indicate a non-sib-cross, we can represent the eight crosses of each series as follows:

♀ (L-1) × ♂ S (L-20),	♀ (H-9) × ♂ S (L-20),
♀ (L-1) × ♂ S (H-31),	♀ (H-9) × ♂ S (H-31),
♀ (L-1) × ♂ C (L-88),	♀ (H-9) × ♂ C (L-88),
♀ (L-1) × ♂ C (H-98),	♀ (H-9) × ♂ C (H-98).

Pollinations of all crosses of a series were completed as a rule within a total period of 2 hours. One head of each set of three was fixed in CRAF fluid 48 hours after pollination; 30 ovaries from each of these heads were sectioned at 15 μ, stained in Heidenhain's iron hematoxylin, and examined for data on fertilization and early development. The other two heads, of each set of three, were allowed to mature until about 30 days after pollination when they were examined for the percentages of florets setting seed. Eventually, two such series were completed for family 1 (see Table I, crosses 11–18 and 21–28), two for family 2 (crosses 31–38 and 41–48), one for family 3 (crosses 51–58), and one for family 4 (crosses 61–68).

An ovule was classified as fertile, that is, as having an apparently normal mature embryo sac, only if the egg, polar fusion nucleus (or two polar nuclei), and synergids were present. The percentage of fertile ovaries (ES) was determined as the percentage of ovaries containing at least one mature embryo sac. An ovule was considered to be developing when there was clear evidence of fertilization such as a developing embryo and endosperm (Figs. 1, 2) and as non-developing when at the time of fixation (*a*) it contained no embryo sac or an immature one (Fig. 3), (*b*) it was reduced in size and contained only signs of embryo-sac formation (Fig. 6), (*c*) it was somewhat reduced in size with a degenerated nucellus (as the smaller ovule in Fig. 5), (*d*) it contained a necrotic

TABLE I

Percentages of ovaries (florets) containing mature embryo sacs, developing ovules, and seed

Plant types crossed	Family 1						Family 2						Family 3, crosses 51–58			Family 4, crosses 61–68		
	Crosses 11–18			Crosses 21–28			Crosses 31–38			Crosses 41–48								
	ES	DO	SS	ES	DO	SS	ES	DO	SS	ES	DO	SS	ES	DO	SS	ES	DO	SS
	(♀ parent L-1)			(♀ parent L-2)			(♀ parent L-3)			(♀ parent L-6)			(♀ parent L-4)			(♀ parent L-5)		
L × SL	[3.3	0.0	1.0]	30.0	13.3	9.0	[21.1	0.0	0.0]	[84.2	0.0	0.0]	[55.0	0.0	0.0]	70.0	53.3	9.4
L × SH	[6.7	6.7	3.2]	43.3	33.3	12.5	[20.0	0.0	0.0]	82.7	58.6	8.0	40.0	23.3	12.6	[63.2	0.0	0.0]
L × CL	[0.0	0.0	3.2]	40.0	23.3	15.9	53.3	23.3	1.2	86.2	44.8	3.1	60.0	3.3	1.1	72.4	48.3	26.0
L × CH	[3.3	3.3	2.3]	24.1	13.8	9.2	70.0	36.7	0.0	71.4	64.3	6.1	53.1	46.9	11.6	70.0	53.3	14.3
	(♀ parent H-9)			(♀ parent H-8)			(♀ parent H-12)			(♀ parent H-7)			(♀ parent H-11)			(♀ parent H-10)		
H × SL	[82.1	3.5	24.0]	[80.0	0.0	0.0]	100.0	76.7	82.1	80.0	73.3	36.7	95.5	95.5	76.6	93.3	90.0	86.0
H × SH	87.5	75.0	91.4	[95.0	0.0	0.0]	[100.0	0.0	1.0]	[78.9	0.0	0.0]	[96.4	0.0	87.6]	93.3	10.0	6.0
H × CL	88.6	85.7	95.4	70.0	63.3	71.0	100.0	36.7	19.2	77.9	51.8	14.4	[100.0	23.3	61.7]	73.3	70.0	96.0
H × CH	100.0	96.7	88.0	96.7	96.7	81.2	100.0	96.7	83.9	70.0	70.0	33.7	100.0	93.1	88.3	100.0	100.0	90.2

NOTE: Crosses are numbered from top to bottom in each column; L = low yield, H = high yield, S = sib, C = non-sib.
ES = percentage of ovaries containing at least one mature embryo sac, i.e. percentage of fertile ovaries.
DO = percentage of ovaries containing at least one fertilized and developing ovule, i.e. percentage of developing ovaries.
SS = percentage of florets (ovaries) containing at least one seed, i.e. percentage of florets setting seed.
Crosses in brackets = incompatible crosses (Nos. 25, 26, 31, 32, 36, 41, 46, 51, 62), technical error (15, 56, 57), irregular meiosis (11, 12, 13, 14).
Numbers following ♀ parents refer to plants as listed in Table 2 of our previous report (12).

embryo as in Fig. 4. The percentage of developing ovaries (DO) was determined as the percentage of ovaries containing at least one developing ovule. The percentage of florets setting seed (SS) was determined as the number of ovaries containing at least one seed about 30 days after pollination.

2. Fertilization and Early Ovule Development

The data on fertilization, early ovule development, and seed set are summarized in Table I. It should be noted that the female parent of crosses 11–14 inclusive had some meiotic disturbances. This was apparently the cause of the consistently low percentages of fertile ovaries, developing ovaries, and florets setting seed in the crosses mentioned. A good number of sib-crosses were found to be incompatible, apparently because the plants crossed are of the same genotypes for the S alleles (crosses 25, 26, 31, 32, 36, 41, 46, 51, and 62), i.e. 9 crosses out of 24. No incompatible combinations were observed in the out-crosses. It is believed that in crosses 15, 56, and 57 some technical or sampling error was involved since it is not possible to get a higher percentage of seed set than of developing ovaries.

An analysis of variance was done on the complete set of data after transformation into angular units and a summary of the analysis is shown in Table II and the group means of observed values in Table III. These results show that, on the average, the proportions of developing ovaries (DO) and

TABLE II

Summary of analysis of variance of the data in Table I after they were transformed into angular units

Source	D.F.	*F*			
		DO	SS	.05	.01
Relationship (sibs vs. out-crosses)	1	12.90**	6.41*	4.11	7.39
Seed set (SS) in ♀ parent	1	13.06**	45.84**		
Seed set (SS) in ♂ parent	1	—	—		
Relationship × SS ♀ parent	1	2.20	3.00		
Relationship × SS ♂ parent	1	6.01*	1.23		
Seed set ♀ parent × SS ♂ parent	1		2.33		
Replications (series)	5		1.97	2.48	
Error	36				
Total	47				

*Significant at the 0.05 level.
**Significant at the 0.01 level.

TABLE III

Means of observed values for Table I

		Percentages of:	
		DO	SS
Type of cross:	Sib-crosses	25.5	22.8
	Out-crosses	51.9	38.2
Seed yield in ♀ parents:	♀ L parent	22.9	6.2
	♀ H parent	54.5	54.7
Seed yield in ♂ parents:	♂ L parent	36.6	30.5
	♂ H parent	40.8	30.4

seed set (SS) were higher in the combined out-crosses than in the combined sib-crosses and the differences were significant at the 0.01 and 0.05 levels respectively. The corresponding means for DO and SS were much higher in the crosses where the female parents were low-seed-yielding and both the differences were significant at the 0.01 level. The high significance of the difference in seed set is, of course, to be expected since the plants used in the crosses were initially selected on a seed-yield basis. Other comparisons and interactions, with one exception, were not significant and the lack of significance of the interactions tested is indicative of a regular relationship between the characteristics tested.

The significance obtained in the general analysis probably does not warrant a firm conclusion that the out-crosses are better than the sib-crosses in percentage of developing ovaries and in percentage of florets setting seed since only one subdivision (♀H ♂H) out of four gave highly significant differences and the differences in the three remaining subdivisions (♀L ♂L, ♀L ♂H, and ♀H ♂L) were not significant.

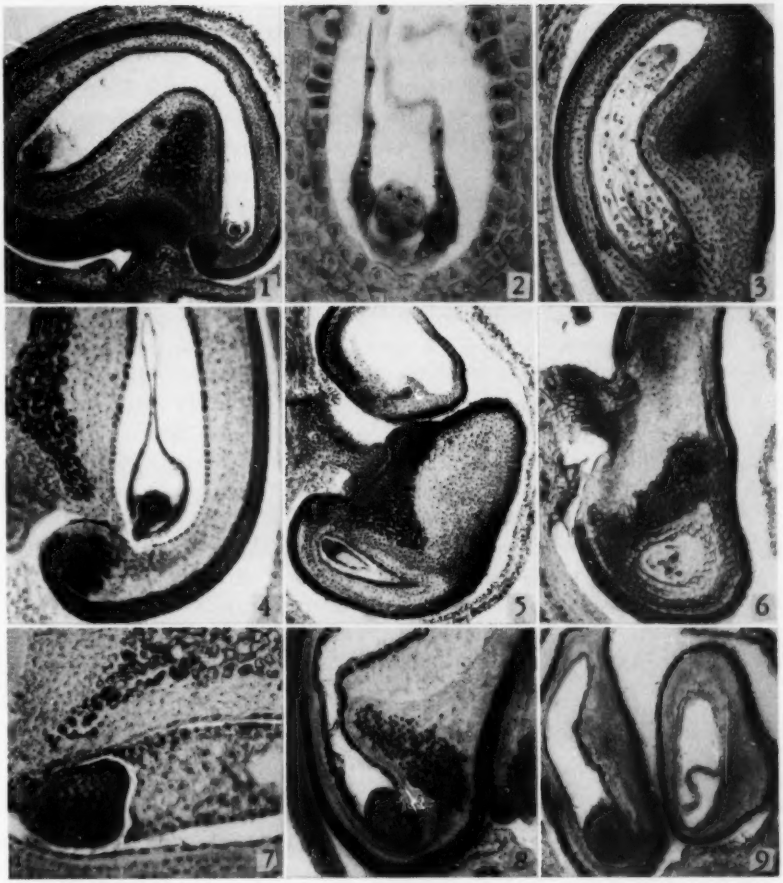
The general analysis showed no significant differences in percentages of developing ovaries and in percentages of florets setting seed as determined by the pollen coming from high- vs. low-seed-yielding plants.

The ratios DO/ES, SS/ES, and SS/DO in Table IV were also calculated for all the crosses from the percentages given in Table I, except those in parentheses. The ratio DO/ES can be defined as the proportion of fertile ovaries that become developing ovaries; the ratio SS/ES is then the proportion of fertile ovaries that produce at least one seed; and the ratio SS/DO is the proportion of developing ovaries that produce at least one seed. The percentage of fertile ovaries (florets) is a limiting factor on both the percentage of developing ovaries and the percentage of ovaries (florets) setting seed, whereas the percentage of developing ovaries is only a limiting factor on the percentage of florets setting seed.

A comparison of the means of these ratios in Table IV reveals that in the combined ♀L crosses only 60.7% of the fertile ovaries showed postfertilization development in at least one ovule and that only 18.9% of the fertile ovaries developed at least one seed. Also, only 31.8% of the developing ovaries in the combined ♀L crosses produced a mature seed. In the combined ♀H crosses, however, 85.0% of the fertile ovaries contained at least one developing ovule after fertilization, and 72.8% of fertile ovaries produced a seed. Also, 81.7% of developing ovaries matured a seed in these crosses. Thus the ♀L plants produced fewer fertile ovaries, fewer of which became developing ovaries, and fewer of their developing ovaries produced seed than did the ♀H plants grown under the same general environment.

Correlation coefficients between the following pairs of data were also calculated for all crosses in Table I except those in parenthesis and found to be:

	♀L	♀H
DO/ES and SS/ES	0.200	0.795***
SS/ES and SS/DO	0.942***	0.923***
DO/ES and SS/DO	−0.104	0.526*



FIGS. 1–9. Normal and abnormal ovule development in diploid red clover.
FIG. 1. Normal embryo and endosperm 2 days after pollination. ×50
FIG. 2. Normally developing embryo and endosperm, 2 days. ×310
FIG. 3. Aborted nucellus: no embryo sac, 2 days. ×95
FIG. 4. Necrotic embryo, normal endosperm, 4 days. Pollen tube in the micropyle. ×95
FIG. 5. One ovule developing normally, the other aborting, 4 days. ×50
FIG. 6. Aborting ovule: remnants of embryo sac still visible, 4 days. ×95
FIG. 7. Normal embryo: cellular endosperm in the micropylar end of the nucellar cavity, 4 days. ×75
FIG. 8. Aborting ovule: embryo large with vacuolated cells; endosperm degenerated: integumental tepetum still well stained; 10 days. ×50
FIG. 9. Two aborted ovules: one with vacuolated embryo, no endosperm, and only remnants of the integumental tapetum; 10 days. ×50

In the first line we correlate the percentages of developing ovaries (or the secondary limiting factor on seed set) and the percentage of fertile ovaries setting seed (or the end result) through the medium of the percentage of fertile ovaries (or the primary limiting factor on seed set). The high correlation in the ♀ H category shows that there is a definite relationship between the percentages of developing ovaries and the percentage of ovaries setting seed. The low and not significant coefficient in the ♀ L category indicates that the percentage drop from DO to SS is not consistent in the different crosses and may indicate the action of certain factors which did not manifest themselves in the ♀ H category. The pair of coefficients in the second line show in effect the relation between both limiting factors (ES and DO) as reflected on percentages of ovaries setting seed. The correlation coefficients are highly significant in both the ♀ L and ♀ H categories. This is to be expected; ES and DO are related to some extent, the former (ES) being a limiting factor on the latter (DO). The two coefficients in the third line are between two apparently independent sets of ratios, that is, between the ratios of the two limiting factors (ES and DO) and the ratios of the result (SS) and the secondary limiting factor (DO). The correlation coefficient is small and not significant in the ♀ L category and significant at the 0.05 level in the ♀ H category. These low coefficients suggest that the factors causing the numerical drop from ES to DO and from DO to SS do not express themselves uniformly in the different crosses or that the factors acting at these two stages are not the same.

The relationship between developing ovules and available embryo sacs is illustrated below using only data for the strictly comparable sets of crosses L × CL (23, 33, 43, 63), H × CL (27, 37, 47, 67), L × CH (24, 34, 44, 64), and H × CH (28, 38, 48, 68).

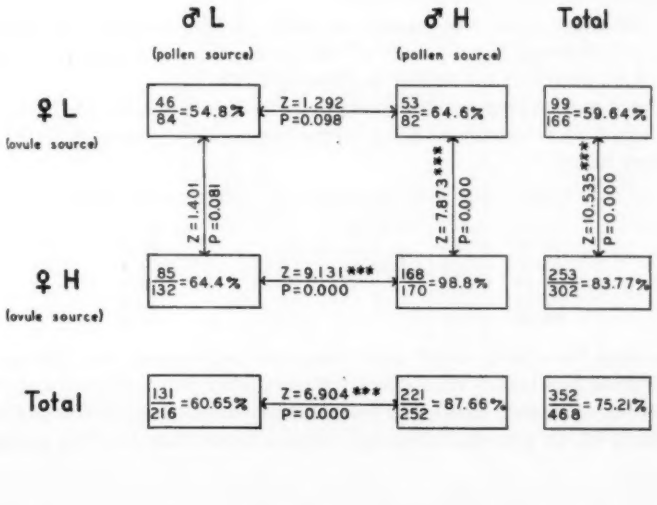


TABLE IV

The means of the various ratios between ES, DO, and SS

	♀ Low-seed-yielding	♀ High-seed-yielding
DO/ES	60.7	85.0
SS/ES	18.9	72.8
SS/DO	31.8	81.7

The actual figures for the fractions, developing ovules over available ovules, are given with the z and p values for the differences based on an Arcsin test. Apparently a few more ovules of ♀L plants start to develop if the pollen comes from an H plant rather than an L plant, but many more ovules of ♀H plants start when the pollen source is an H plant instead of an L one. Also it seems that pollen of L plants is only slightly more effective on H plants than on L ones whereas pollen of H plants is much more effective on H plants than on L plants.

In the above sets of crosses the proportions of developing ovules maturing to seed were: ♀L♂L 3/46 = 6.5%; ♀L♂H 2/53 = 3.8%; ♀H♂L 21/85 = 24.7%; and ♀H♂H 61/168 = 36.3%. Clearly more ovules matured in ♀H plants than in ♀L plants regardless of pollen source. Pollen of H plants contributed no advantages over that from L plants regarding seed set on ♀L plants but seems to have done so on the ♀H plants ($P=0.057$ in an Arcsin test) and clearly so in the combined ♀L + ♀H total ($P=0.028$). This latter advantage was not apparent in the general analysis of variance (Table II) where comparisons are less dependable. It appears that low-seed-producing plants are less capable of supporting ovule development and that pollen of high-seed-producing plants somehow enhances the ability of ovules to develop into seed.

3. Embryo and Endosperm Development

In this experiment, the number of cells per embryo and the number of nuclei per endosperm were counted in the developing ovules in all possible cases. A summary of the results is given in Table V.

Embryo and endosperm development at 2 days in crosses 11–18 was found to be much more advanced than in the remaining crosses, as shown in the summary below.

Crosses	Cells per embryo	Nuclei per endosperm
11–18	15.1	102.9
51–58	8.3	69.8
31–38	10.8	59.7
41–48	8.0	52.9
21–28	7.0	51.3
61–68	5.8	42.1

Pollinations for crosses 11–18 were completed before noon and the maximum temperature was higher that afternoon than after pollinations for any of the other series of crosses. The mean temperature for the 2 days after pollinations for crosses 61–68 was six Fahrenheit degrees lower than for that period after

TABLE V

Embryo and endosperm development 48 hours after pollination in crosses of diploid red clover in 1954

Plant types crossed	Family 1						Family 3						Family 2, crosses 51–58			Family 4, crosses 61–68			Means (weighted)		
	Crosses 11–18			Crosses 21–28			Crosses 31–38			Crosses 41–48											
	No.	Emb.	End.	No.	Emb.	End.	No.	Emb.	End.	No.	Emb.	End.	No.	Emb.	End.	No.	Emb.	End.	No.	Emb.	End.
L × SL	—	—	—	4	7.5	44.2	—	—	—	—	—	—	—	—	—	16	4.5	32.8	20	5.3	35.0
L × SH	2	14.5	58.5	10	6.8	38.7	—	—	—	16	4.8	28.6	7	6.1	38.3	—	—	—	35	6.2	35.1
L × CL	—	—	—	7	9.9	60.4	4	8.2	37.5	12	8.4	60.3	1	8.0	44.0	14	5.4	22.4	38	7.6	43.6
L × CH	1	12.0	71.0	3	5.7	35.7	10	6.5	36.2	14	9.2	64.2	15	11.2	93.5	16	4.4	25.2	59	7.8	55.0
H × SL	1	7.0	63.0	—	—	—	36	11.4	69.0	23	7.5	53.2	23	8.1	51.3	36	6.8	51.2	119	8.6	57.1
H × SH	35	11.1	84.1	—	—	—	—	—	—	—	—	—	—	—	—	1	2.0	4.0	36	10.8	81.9
H × CL	37	12.4	101.4	29	6.5	51.6	11	11.9	70.1	14	9.2	58.4	5	6.4	83.8	28	6.5	50.0	124	9.0	69.8
H × CH	45	20.7	122.4	44	7.1	56.5	46	11.3	56.9	22	9.2	55.5	35	7.9	76.8	42	5.9	46.4	234	10.6	70.4

NOTE: Crosses are numbered from top to bottom in each column; see Table I for other notes.
No. = number of ovules.
Emb. = average number of cells per embryo.
End. = average number of nuclei per endosperm.

crosses 11–18, and the second day was so rainy and cloudy that the maximum temperature dropped to the lowest level during any of these crosses. Since there were many cells per embryo and nuclei per endosperm in crosses 11–18 and relatively very few in crosses 61–68, it seems that any one or more of pollen germination, pollen tube growth, cell division in the embryo, and nuclear division in the endosperm may be accelerated at higher temperatures.

Since the number of embryos (and endosperms) examined in ovaries involved in the different crosses varied greatly, it was only possible to make a simple analysis of variance (between–within), a summary of which is given in Table VI. The mean number of cells per embryo and the mean number of nuclei per endosperm for the major groupings of crosses are presented in Table VII. Both embryo and endosperm development were more advanced in the ♀H group than in the ♀L (Table VII) and the differences were highly significant (Table VI). The same level of significance of the differences was found in direct comparisons of ♀H and ♀L within the same background in the four subdivisions (♀L♂L, ♀L♂H, ♀H♂L, ♀H♂H). It seems, therefore, fairly safe to conclude from the data obtained that both embryos and endosperms were more advanced in the high-seed-producing female plants than in the low-seed-producing ones.

Both embryos and endosperms were more advanced in the out-crosses than in the sib-crosses and the differences were highly significant in the over-all

TABLE VI

Summary of analysis of variance for embryo and endosperm development in crosses of diploid red clover in 1954

Source	D.F.	*F*			
		Embryos	Endosperms	.05	.01
Between treatments	7	7.81**	9.24**	2.02	2.66
♀ L vs. ♀ H	1	40.12**	41.74**	3.85	6.66
♂ L vs. ♂ H	1	11.20**	4.63*		
Sib-cross vs. out-cross	1	9.45**	10.45**		
Within treatments	657				
Total	664				

TABLE VII

Means of the major groupings of crosses for embryo and endosperm development in diploid red clover in 1954

	Mean number of:	
	Cells per embryo	Nuclei per endosperm
♀ Low-seed-yielding	7.01	44.94
♀ High-seed-yielding	9.79	67.96
♂ Low-seed-yielding	8.40	59.16
♂ High-seed-yielding	9.77	65.63
Sib-crosses	8.23	55.58
Out-crosses	9.58	65.98

analysis. However, the direct comparisons of sib- and out-crosses within the common background in the above-mentioned four subdivisions showed a somewhat confused distribution of significances concerning both embryos and endosperms. A non-parametric test (17) of the combined sib-cross vs. out-cross comparisons reveals a significant difference between endosperms but not between embryos. It seems that the out-crosses have some advantages in endosperm development.

The embryos were more advanced in the ♂H group than in the ♂L, if we accept the highly significant difference in the over-all analysis, but the corresponding difference in endosperm development was only significant at the 0.05 level. Since most direct comparisons of ♂L and ♂H for both embryo and endosperm within the four subdivisions mentioned above were not significant, we cannot claim to have demonstrated that the pollen source has an immediate effect upon embryo and endosperm development even though there is a suggestion that it may have an effect. A non-parametric test (17) revealed no significant differences regarding embryo or endosperm, thus supporting this attitude, but it should be noted that the data suggest a slight advantage for pollen from H plants. This is consistent with the previously noted advantage of such pollen in the initiation of ovule development and in the development of ovules to maturity.

In the six H × CH crosses, 98 ovaries having both ovules developing were found and the following regression coefficients have been calculated for them from the observed values:

(1) Regression of the number of nuclei per endosperm on the number of cells per embryo

(a) in the upper ovules $4.762 \pm 0.401^{**}$,

(b) in the lower ovules $5.853 \pm 0.391^{**}$.

(2) Regression of the number of cells per embryo of the lower ovules on the upper ovules $0.650 \pm 0.049^{**}$.

(3) Regression of the number of nuclei per endosperm of the lower ovules on the upper ones $0.573 \pm 0.073^{**}$.

The first two coefficients (1a and 1b) show that at this early stage of development the endosperm grows relatively more rapidly than the embryo, that is, for each cell division in the embryo several nuclear divisions occur in the endosperm. The difference between these two coefficients (just missing the 0.05 level of significance) should mean that the upper ovules are on the average more advanced than the lower ones. The last two regression coefficients tend to confirm this statement and the following data (means of the 98 ovaries) lend further support to it.

	Mean number of cells per embryo	Mean number of nuclei per endosperm
Upper ovules	9.37	59.41
Lower ovules	7.81	53.74

Apparently, embryo and endosperm start to develop in the upper ovules first, as might be expected.

Experiment II. Ovule Development at Stages between Pollination and Seed Maturation following Outcrossing of High- and Low-Yielding Red Clover Plants

1. Materials and Methods

In this experiment it was initially planned to make a direct comparison of ovule development in high- and low-yielding plants (H and L) outcrossed to a common high-yielding pollen parent (H) that produced a high percentage of good pollen. We hoped to produce six series each consisting of the two crosses ♀ (L-1) × ♂ C (H-1) and ♀ (H-2) × ♂ C (H-1), using the same letter system as in experiment I. However, we had a severe winter in 1954/1955 and lost most of the plants for which the pertinent information was available. We then decided to proceed in 1955 by using early heads for a quick check of seed-yielding ability and by making on this basis what we hoped would prove to be acceptable series on the above plan later when we had more adequate data on the seed yields of the plants involved. We succeeded in completing four series (tests 3–6 inclusive in Tables VIII and IX) but we had only high-yielding female parents in the other two series. We will refer to these series as tests.

The 10 plants chosen as female parents in the crosses of the first five tests had themselves a common female parent, and the first four of them had also a common pollen parent which was a sib of the common pollen parent of the last six. The two other female parents, used in the sixth test, were sibs in a different but related progeny. The pollen parents used in all the crosses of all six tests were all sibs (from progeny A, see ref. 12) and fairly closely related in a general way to all the plants used as female parents in the crosses of these tests. The data on seed yield etc. for these parental plants are contained in Table VIII.

TABLE VIII

Characteristics of the parental plants used in the ovule development tests in 1955

		Female parent				Male parent		
Test No.	Cross	Yield rating	% florets setting seed O.P.	% fertile ovaries (E.S.)	% good pollen	Yield rating	% florets setting seed O.P.	% good pollen
1	A1	H	40.5	77.8	69.4	H	69.1	95.8
	A2	H	75.6	96.6	97.6			
2	B1	H	60.0	93.1	95.1	H	82.5	96.0
	B2	H	79.4	96.4	46.0			
3	C1	L	22.9	83.8	92.0	H	83.8	96.6
	C2	H	84.5	100.0	51.1			
4	D1	L	28.9	83.8	87.6	H	74.3	97.9
	D2	H	70.7	96.8	94.5			
5	E1	L	3.2	39.4	91.0	H	75.4	94.4
	E2	H	86.3	87.5	94.6			
6	F1	L	50.8	45.8	59.6	H	79.9	95.0
	F2	H	89.3	100.0	95.0			

On each plant being used as a female parent five heads with mature but unopened flowers were selected and covered with cellophane bags. Two days later the bags were removed, and the five heads were trimmed to 30–50 florets and cross-pollinated as indicated in Table VIII before they were again covered with new bags. One head, at each of the stages 2, 4, 7, and 10 days after pollination, was prefixed for 30 seconds in 3:1 alcohol–acetic solution before fixation in CRAF fluid. The fifth head was examined 30 days after pollination to obtain data on the percentage of florets setting seed. Ovaries collected at 2 and 4 days after pollination were sectioned at 15 μ and stained in Heidenhain's iron hematoxylin whereas those collected at 7 and 10 days were dissected out and examined under a dissecting microscope with direct illumination. Some of the doubtful cases at 7 and 10 days were later sectioned and stained for further examination. About 30 ovaries were examined at each stage up to 10 days for each cross.

Ovaries and ovules were classified as in experiment I. Each ovary was classified, as *00* where neither of the two ovules contained a mature embryo sac, as *01* where only one of the two ovules contained such an embryo sac, and as *11* where both ovules contained such an embryo sac. This classification was extended to classify ovaries with respect to developing ovules and to those setting seed. A new type of non-developing ovule, containing a vacuolated embryo and absorbed endosperm (Figs. 8 and 9), was added to the abnormal types previously listed. Other conditions of ovules at later stages are illustrated in Figs. 3 to 7.

2. General Consideration of Data regarding Embryo Sac Production and Early Development

The results of this experiment, shown as percentages of the 30 ovaries examined at each stage and of the entire heads at maturity, are presented in Table IX. It should be kept in mind that the distributions of classes of ovaries regarding mature embryo sacs and regarding developing ovules at 2 days were determined simultaneously from the same ovaries sectioned at 2 days after pollination. The use of a common pollen source for the two plants used as female parents in each test makes it possible to compare fertilization and developmental processes in the ovules of the two types of seed-yielding plants. For the direct comparison of H and L female parent plants, however, we are restricted, of course, to tests 3 to 6 inclusive. Also in making any comparisons at particular stages of development it is well to keep in mind that the sample is really small and that variations between heads on the same plant probably also exist.

Individual plants varied a great deal in their production of mature embryo sacs. Female parents in crosses C2 and F2 for example produced only fertile ovaries in this respect whereas those of crosses E1 and F1 produced a majority of ovaries that lacked normal embryo sacs even at this late stage and, accordingly, were unable to produce much seed for this reason alone. On the other hand it is clear from results of crosses C1 and D1 that the ability to produce

plenty of mature embryo sacs by no means ensures high fertility in terms of seed set. It is generally apparent in these data, however, that reduced production of normal embryo sacs by a plant places a primary restriction on the potential seed yield of that plant.

TABLE IX

Distribution of classes of ovaries for percentages of mature embryo sacs and ovule development, 1955

Test No.	Cross	Type of cross	Class of ovary	Percentages of ovaries of each class					
				For ES (2 days)	For development at: (days)				
					2	4	7	10	30 (seeds)
1	A1	H × H	*00*	22.2	38.9	36.7	14.3	25.0	16.7
			01	55.6	44.4	63.3	80.9	75.0	83.3
			11	22.6	16.7	0.0	4.8	0.0	0.0
	A2	H × H	*00*	3.4	10.3	30.0	0.0	20.0	20.0
			01	24.1	37.9	70.0	91.4	80.0	80.0
			11	72.5	51.8	0.0	8.6	0.0	0.0
2	B1	H × H	*00*	6.9	48.3	24.1	18.4	15.8	30.4
			01	34.5	37.9	75.9	63.2	81.6	58.7
			11	58.6	13.8	0.0	18.4	2.6	10.9
	B2	H × H	*00*	3.6	21.4	40.0	4.8	2.5	5.7
			01	42.8	60.7	60.0	95.2	97.5	94.3
			11	53.6	17.9	0.0	0.0	0.0	0.0
3	C1	L × H	*00*	16.2	71.0	34.4	58.3	75.7	85.0
			01	41.9	25.8	56.2	27.8	18.2	15.0
			11	41.9	3.2	9.4	13.9	6.1	0.0
	C2	H × H	*00*	0.0	24.2	6.7	17.1	20.9	30.2
			01	16.0	33.3	43.3	78.0	74.5	69.8
			11	84.0	42.5	50.0	4.9	4.6	0.0
4	D1	L × H	*00*	16.2	77.4	58.6	64.3	64.5	71.1
			01	38.7	22.6	37.9	35.7	35.5	28.9
			11	45.1	0.0	3.5	0.0	0.0	0.0
	D2	H × H	*00*	3.2	41.9	29.0	36.4	25.0	24.4
			01	38.7	45.2	41.9	59.1	75.0	75.6
			11	58.1	12.9	29.1	4.5	0.0	0.0
5	E1	L × H	*00*	60.6	72.7	75.9	76.2	85.0	100.0
			01	39.4	27.3	24.1	23.8	15.0	0.0
			11	0.0	0.0	0.0	0.0	0.0	0.0
	E2	H × H	*00*	12.5	15.6	6.2	22.2	25.0	20.0
			01	34.4	34.4	75.0	73.4	75.0	80.0
			11	53.1	50.0	18.8	4.4	0.0	0.0
6	F1	L × H	*00*	54.2	58.3	50.0	38.5	45.0	66.7
			01	45.8	41.7	50.0	61.5	55.0	33.3
			11	0.0	0.0	0.0	0.0	0.0	0.0
	F2	H × H	*00*	0.0	5.9	9.4	6.0	6.7	7.5
			01	11.8	26.5	71.9	94.0	93.3	92.5
			11	88.2	67.6	18.7	0.0	0.0	0.0
Means		L × H	*00*	36.8	69.9	54.7	59.3	67.6	80.7
			01	41.4	29.3	42.1	37.2	30.9	19.3
			11	21.8	0.8	3.2	3.5	1.5	0.0
Means		H × H	*00*	6.5	25.8	22.7	14.9	17.6	19.4
			01	32.2	40.0	62.7	79.4	81.5	79.3
			11	61.3	34.2	14.6	5.7	0.9	1.3

There is evidence that fertilization and/or early development were delayed in several crosses. For example, in B1, C1, D1, and D2 many available ovules were not developing at 2 days. In C1 and D1 the low-yielding female parents involved in this delay never did overcome it, which is apparently an important factor regarding their low seed yields. In contrast the high-yielding female parents of crosses A2 and B2 were able to overcome similar, but less extensive, delays in the initiation of development and to produce plenty of seed.

Since the tests were completed on different days, variations in temperature following pollination may have contributed to the differential success of fertilization and/or early development in the various crosses. The *differences* in frequencies of ovary development between 2 and 4 days after pollination were correlated with the mean temperatures, mean minimum temperature, and mean maximum temperature, for the 2 days after pollination. The correlations obtained were -0.537, -0.449, and -0.602, respectively, only the last being significant and at the 0.05 level. The negative signs of these correlations indicate that increasing temperatures, in these limits, elevated the percentages of fertilized ovules and/or the initiation of development before 2 days. Plants of tests 3 and 4 were pollinated during a cold spell and show delayed initiation of development. Warm weather followed pollination of test 5 plants and accompanied earlier initiation of development but similar weather after pollination for test 2 accompanied delay. Also, female plants of test 2 pollinated at nearly the same time seem to differ in the duration and extent of the delay in development. It seems not only that lower temperatures delay these initial processes but also that individual plants vary in their responses to such environmental influences.

The means at the bottom of Table IX provide a comparison between development in high-yielding (H) and low-yielding (L) female parents when both types are crossed with high-yielding plants that produce abundant pollen. It will be noticed, however, that the means for L × H crosses are calculated for crosses of tests 3 to 6 inclusive, whereas those for the H × H crosses are for all tests. Since crosses of tests 1 and 2 are not completed under the strictly comparable conditions prevailing for the H and L female parents in the other tests it is obviously preferable to restrict the H vs. L comparison to the last four tests. An analysis of variance of the percentages of developing ovaries (*01* + *11* classes of Table IX) at the five developmental stages in these

TABLE X

Summary of analysis of variance of the percentages of developing ovaries at different periods after pollination after transformation into angular units

Variation due to:	D.F.	M.S.	*F*	.05	.01
Yielding ability	1	9388.09	175.97**	4.21	7.68
Periods	4	172.32	3.23*	2.73	4.11
Replications (tests)	3	462.54	8.67**	2.96	4.60
Yielding ability × periods	4	69.35	1.30		
Error	27	53.35			
Total	39				

four tests, transformed into angular units, is summarized in Table X. The same percentages (not transformed) have been used in preparing the histograms shown in Fig. 10.

The analysis of variance reveals highly significant differences between H and L plants, significant differences between periods, highly significant differences between tests (tests 3, 4, 5, and 6), and non-significant interaction between yielding ability × periods. The latter result simply shows that H and L plants responded in the same way at all periods.

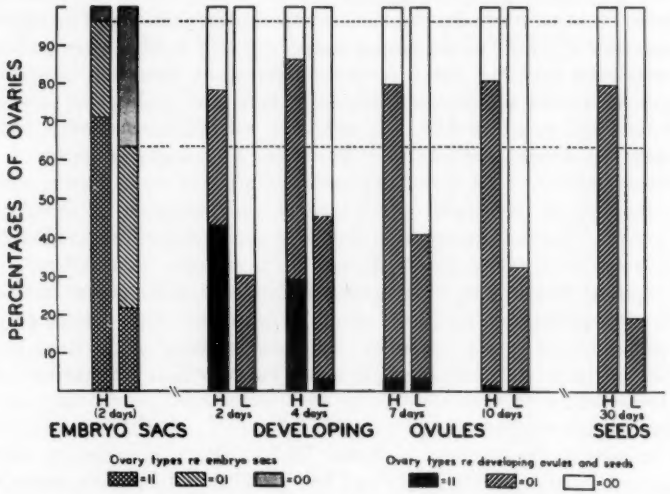


FIG. 10. Frequencies of embryo sacs, developing ovules, and seeds in ovaries of high (H) and low (L) seed-yielding plants of red clover at stages of ovule development.

The graphic representation of the same results in Fig. 10 provides a clear picture of the differences between H and L plants. The H plants have many *11* ovaries (70.9%) and some *01* ovaries (25.2%) regarding production of mature embryo sacs so they have high potential seed-producing ability. This potential (96.1%) sets the limit of development at all later stages as shown by the dotted line extending to the right in the figure. The L plants produce few *11* ovaries (21.8%) and some *01* ovaries (41.4%) so that potential seed set is thereby restricted to 63.2% as shown by the lower dotted line extending to the right in the figure. Though individual plants differ within both H and L categories, the two categories differ strongly in general.

Fertilization in the H category plants is highly successful, judged on the basis of initial development, though apparently not completed at 2 days. The percentage of developing ovaries (i.e. those containing at least one developing ovule) reaches about 90% of the potential at 4 days and this level drops only slightly at later stages of maturity. In some ovaries fertilization is not achieved in both ovules though embryo sacs are available in both, and in many other

ovaries competition between the two developing ovules results in the abortion of one before 10 days of development. The latter losses of ovules are normal and do not reduce the expected seed yields, and furthermore the H plants support the remaining *01* ovaries so well that nearly all of them produce a seed.

In contrast, fertilization in the L category plants is achieved less frequently and is apparently more delayed. The percentage of developing ovaries, even at its highest level at 4 days, is just over 70% of the potential and drops rapidly in later stages of maturity. Even when both ovules of an ovary contain embryo sacs, development is seldom initiated in more than one of them. Since only one ovule starts to develop in less than half of the ovaries, and since these plants provide very poor support for even these few *01* ovaries, their seed yields are low.

3. Production of Embryo Sacs and Initiation of Development in Upper and Lower Ovules

With very rare exceptions each ovary contains two ovules, each of which usually contains an embryo sac. In tests 3–6 inclusive the four L plants produced on the average, per ovary, 0.87 ovules containing embryo sacs, whereas the four H plants produced 1.62 such ovules per ovary. The L plants averaged 0.29 developing ovules per ovary at 2 days and 0.50 per ovary at 4 days. The H plants averaged 1.28 developing ovules per ovary at 2 days and 1.33 at 4 days. Thus the high-yielding plants were much superior in initiating development in the available embryo sacs.

A refinement of the classification of *01* ovaries regarding apparently normal embryo sacs in the ovules involves recording the location of the embryo sac as in the upper or lower ovule of each ovary. The ovaries can then be classified as follows:

00 when neither ovule has an embryo sac,
01U when only the upper ovule has an embryo sac,
01L when only the lower ovule has an embryo sac, and
11 when both ovules contain an embryo sac.

This type of classification has also been used for ovaries with respect to the distribution of developing ovules. The pooled data for tests 3–6 inclusive of experiment II classified on these two bases at 2 and at 4 days are assembled in Table XI.

Regarding embryo sacs, the tendencies of low-yielding plants to produce many *00* ovaries and of high-yielding ones to produce a majority of *11* ovaries are again very clear. Also, it seems that *01U* ovaries are produced as often as *01L* ones by both H and L plants. These points were further confirmed using data for H and L plants of experiment I. In fact, since the pollen parent in a cross cannot possibly affect the production of embryo sacs by the female parent plant, it seemed permissible to combine all such data for female parent plants of both experiments and by doing so we get the following totals. Ten L plants produced 444 ovaries of the *00* class (48.5%), 160 of the *01U* class (17.5%),

TABLE XI

Distribution of developing ovules, tests 3–6 inclusive of experiment II

Days after pollination	Ovary class regarding embryo sacs	Yield type ♀ parent	No. of ovaries	Developing ovules per ovary				Total ovaries developing
				Neither	Upper only	Lower only	Both	
Two	ES in neither ovule (*00*)	L	43	43	—	—	—	0
		H	4	4	—	—	—	0
	ES upper ovule only (*01U*)	L	25	11	14	—	—	14
		H	13	2	11	—	—	11
	ES lower ovule only (*01L*)	L	22	12	—	10	—	10
		H	15	7	—	8	—	8
	ES in both ovules (*11*)	L	29	19	6	3	1	10
		H	98	14	18	3	63	84
	Subtotal	L	119	85	20	13	1	34
	Subtotal	H	130	27	29	11	63	103
	Total	L and H	249	112	49	24	64	137
Four	ES in neither ovule (*00*)	L	35	35	—	—	—	0
		H	4	4	—	—	—	0
	ES upper ovule only (*01U*)	L	26	5	21	—	—	21
		H	32	5	27	—	—	27
	ES lower ovule only (*01L*)	L	32	11	—	21	—	21
		H	22	2	—	20	—	20
	ES in both ovules (*11*)	L	19	10	2	2	5*	9
		H	67	5	4	1	57†	62
	Subtotal	L	112	61	23	23	5	51
	Subtotal	H	125	16	31	21	57	109
	Total	L and H	237	77	54	44	62	160

*Including one lower ovule with an aborting embryo.
†Including 10 upper and 11 lower ovules of different pairs each with an aborting embryo,

174 of the *01L* class (19.0%), and 137 of the *11* class (15.0%); 14 H plants produced 101 ovaries of the *00* class (8.7%), 215 of the *01U* class (18.5%), 177 of the *01L* class (15.3%), and 667 of the *11* class (57.5%). In the L plants 51.5% of ovaries contained at least one embryo sac but only 35.4% of the ovules had one, whereas in the H plants these respective values were 91.3% and 74.4%. Hence it seems safe to conclude that the upper ovule has no advantage over the lower one in ability to produce a normal embryo sac and that the H plants produced on the average over twice as many ovules available for fertilization per ovary.

When we compare the proportions of ovules developing in the available ovules that contained an embryo sac at 2 and 4 days we find a difference between L and H female parent plants. (See Table XI and note that percentages derived from these figures are not directly comparable with the non-weighted average of Table IX.) At 2 days the upper ovule was developing in 38.9% of the available ovules in that position in L plants and in 82.9% of those in H plants, whereas the lower ovule was developing in 27.5% of the available ovules in that position in L plants and in 65.5% of those in H plants. These data indicate that fertilization (or initiation of development) was slower in the L plants in spite of the fact that they received the same pollen at almost the same time as the H plants. The lower percentages for lower ovules indicates that fertilization is incomplete at 2 days and some lower ovules have not yet been reached by the pollen tubes. At 4 days the upper ovules were developing in 62.2% of the available ovules in that position in L plants and in 88.9% of those in H plants, whereas the lower ovule was developing in 54.9% of the available ovules in that position in L plants and in 87.6% of those in H plants. There is a definite improvement in the percentages of developing ovules in all four categories at 4 days indicating that fertilization has continued between 2 and 4 days. Taking the 2-day percentages over the 4-day ones we get 62.5% for upper ovules of L plants, 93.2% for upper ovules of H plants, 50.0% for lower ovules of L plants, and 74.7% for lower ovules of H plants. Consideration of these results leads to the conclusion that fertilization is more rapid in H seed parents than in L seed parents under strictly comparable conditions unless the delay follows directly after fertilization which seems unlikely. It appears that the delay in the L plants results in many available ovules never being fertilized or that many are fertilized so late that their collapse is perhaps inevitable in these plants.

The ♀L × CH and ♀H × CH combinations of crosses 21–28, 31–38, 41–48, 51–58, and 61–68 of experiment I provide strictly comparable data and confirming results regarding the conclusions of the last paragraph. Here, at 2 days, the upper ovules were developing in 73.4% (36/49) of available ovules in that position for five L plants and in 89.5% (102/114) of those in five H plants, whereas the lower ovules were developing in 54.2% (32/59) of the available ovules in that position in the five L plants and in 90.3% (104/115) of those in the five H plants. Here fertilization appears to have been at least almost complete at 2 days for the H plants and highly successful in both upper and lower ovules but incomplete and delayed in both upper and lower

ovules of the L plants. The agreement of the results of the two experiments in this respect, and the statistical significance of the differences between the L and H categories here and in the data from experiment II, lead one to the conclusion that pollen tubes are commonly undernourished, handicapped, or inhibited in some way in the styles of low-yielding plants. In these L plants fertilization seems to be delayed and the proportion of ovules that develop is limited.

4. Ovule Collapse

The beginning of ovule abortion is sometimes detectable at 2 days after pollination. It is indicated by an increased vacuolization of cells of the embryos and by reduced staining capacity of endosperm nuclei. In some cases the embryo cells at 4 days and later stain very strongly and are apparently necrotic. The cells of these aborting embryos enlarge (without further division) and even separate in some embryos at later stages. Meanwhile the stainability of nucleoli in aborting endosperm nuclei gradually decreases until only traces of cytoplasm can be found and eventually the endosperm cannot be seen though the embryo may still be present. At this time the nucellar tapetum may still stain distinctly (Fig. 8) but later it also does not stain and even the inner integument, and sometimes also the outer, consists only of dead or dying nearly empty cells (Fig. 9). Sometimes only the empty and reduced integuments remain. Such ovule collapses not only occur regularly as a consequence of competition but also take place where competing ovules are not present in the ovary.

In all crosses of experiment II all developing ovules were recorded as normal or collapsing at both 2 and 4 days. A total of 30 ovules out of 334 developing ones (15.6%) were collapsing, including 11.4% at 2 days, 19.8% at 4 days from equal numbers of ovules. Individual plants averaged from 0% to 37.8% of developing ovules that were collapsing at 2 and 4 days combined. Developing upper ovules were collapsing in 11.1% at 2 days, 16.0% at 4 days for nearly equal numbers, giving a general average of 13.6%. Corresponding values for lower ovules were 11.8%, 24.1%, and 17.9%. It would seem that lower ovules tend to collapse slightly more frequently than upper ones at these early stages.

In tests 3–6 inclusive only one ovule was collapsing in 91 developing ovules for 2 plus 4 days from the four L plants (1.1%) whereas 30 out of 334 (9.0%) were doing so in the four H plants. (Sixty-eight out of 264 developing ovules of the four H plants of tests 1 and 2 were collapsing, so the general figure for all H plants increases to 17.8%.) This difference between L and H plants at that stage may be merely a consequence of slower fertilization in the L plants, in which two competing developing ovules were present in only six ovaries. Between 4 days and maturity 31.7% of developing ovules collapsed in H plants and 60.2% in L plants. Though only about twice as many developing ovules collapsed in L as in H plants the reduction in seed yield is 26.0% for them as compared with 7.7% for the H plants because they had only

3.2% *11* ovaries regarding ovule development at 4 days whereas H plants had 29.2% of such ovaries at that stage. The H plants not only support developing ovules better but also provide fertility insurance by starting development in two ovules per ovary much more frequently.

General Discussion and Summary

The ability of diploid red clover plants to produce mature embryo sacs is a highly variable characteristic ranging in frequency from less than 2% of ovules to 94% in individual plants. Martin (8) has stressed the importance of failure to produce ovules containing embryo sacs, and Pandey (9) found in a small sample that "in 98% of the ovaries there were two normal ovules containing fully mature embryo sacs." We have previously stressed that failure to produce embryo sacs is an important limiting factor on the ability of a red clover plant to produce seed (13). In experiment I, 30% of ovules of low-seed-producing plants contained mature embryo sacs and 75% in the high-seed-producing plants, and the corresponding figures for such fertile ovules maturing to seed were about 13% and 46% respectively. In experiment II, 44% of ovules of low-seed-producing plants contained embryo sacs and 74% in the high-seed-producing plants; and the corresponding figures for such ovules maturing to seed are 23% and 53% respectively. In these two experiments, there is then additional evidence that such ovule sterility reduces the seed-yielding ability of red clover plants.

Like Martin (8), we have noticed that the ability to produce embryo sacs is influenced by environmental conditions, but we have not assessed either the extent or the importance of this influence.

High-seed-yielding plants produce two embryo sacs in most of their ovaries. High- and low-yielding plants produce about equal proportions ($20 \pm \%$) of ovaries having only one embryo sac and in such ovaries the single embryo sac is located about as often in the upper as in the lower ovule. Low-yielding plants fail to produce even one embryo sac in most of their ovaries.

Fertilization is completed in some ovaries before 24 hours after pollination and in most ovules by 4 days. Pandey (9) found it had taken place in about half the ovules at 24 hours after pollination in his material. Martin (8) reported great variation in this respect depending on environmental conditions. In five ♀ H × CH crosses of experiment I 90% of the fertile ovules were developing by 2 days but only 74% in comparable crosses in experiment II at 2 days with an increase to 88% at 4 days (Table XI). Crosses for experiment I were completed in July and for experiment II in September when it was cooler, so our general observations seem to support Martin's contention that environmental conditions affect the fertilization process, and other detailed considerations confirm it. In certain crosses some ovules did not start to develop until after 4 days. Of course one cannot be sure in some cases whether these delays involved slow pollen tube growth, delayed union of egg and sperm, or some other cause, but it seems most likely that slow pollen tube growth is primarily responsible for them.

As shown in Table IV for all crosses of experiment I, 61% of ovaries containing one embryo sac (fertile ovaries) became developing ovaries in low-seed-yielding plants, and 85% in high-seed-yielding plants by 2 days after pollination. In five sets of strictly comparable crosses of this experiment 90% of the fertile ovules started to develop in the high-yielding plants but only 63% in the low-yielding plants. The respective corresponding figures for experiment II, tests 3–6 inclusive, at 2 days were 74% and 33% of the fertile ovules and at 4 days 88% and 58% of the fertile ovules, under conditions that were again strictly comparable for the two types of plants. The differences between the two types of plants are statistically highly significant. These results suggest that the delayed initiation of development of ovules in the low-seed-producing plants resulted in many of the available ovules never developing at all, or that many start to develop so late that their collapse is probably inevitable. Two high-seed-yielding plants, however, overcame such an initial setback and produced abundant seed though none of the low ones did. Though in several instances pollen tubes passed the micropyles of ovules containing mature embryo sacs without entering, as observed by Cooper and Brink (3) in alfalfa, such cases were relatively rare in our material. The simplest explanation seems to be that the pollen tubes are frequently undernourished or inhibited in the styles and/or ovaries of the low-seed-yielding plants. This similarity to the action of S alleles reminds one of the fact that such alleles exist abundantly and act in red clover, as has been well established through the work of Williams and his co-workers (14, 15, 16) and more recently of Pandey (9, 10, 11). It seems possible that certain genes in these low-yielding plants act like weak self-incompatibility genes.

In experiment I embryos of low-seed-yielding plants had 7 cells and the endosperms 45 nuclei whereas in high-seed-yielding plants the corresponding figures were 10 and 68, respectively. There was evidence that temperature differences and other weather conditions affected both the number of cells per embryo and the number of nuclei per endosperm. Also there was considerable variation between crosses and plants in respect to both of these indices of development. It does seem safe, however, to conclude from the data obtained that both embryos and endosperms were more advanced in the high-seed-yielding plants than in the low ones. Similarly Das (5) observed that in *Melilotus alba* the highest-fertility line showed the highest rate of early embryonic growth. Furthermore Cooper, Brink, and Albrecht (4) found that in alfalfa "the rate of growth of the embryos in the high seed-setting group is much higher than that in the low seed-producing set of plants." Our difference at this stage of development seems to be a result of earlier fertilization and initiation of development in the high-yielding plants rather than a result of more rapid nuclear and cell divisions in their embryos and endosperms. Of course, high-yielding plants may be superior to low ones in both respects.

Pollen from high-seed-yielding plants seems to have some advantage over that from low ones. In the four sets of strictly comparable crosses in experiment I, 88% of fertile ovules started to develop after application of pollen from high-seed-yielding plants but only 61% when it came from low ones.

In these crosses there was no statistically significant increase in the number of cells per embryo and of nuclei per endosperm following use of pollen from H plants rather than L ones, though the general analysis (Table VI) suggested there might be. On the other hand in these sets of crosses seed production was significantly increased when the pollen came from H plants instead of L ones. This result is interesting in connection with our earlier finding (12) that seed yields of progenies from High × High crosses were significantly superior to those from High × Low crosses.

There is a suggestion in experiment I, again not statistically significant in detailed comparisons, that in out-crosses more fertile ovaries start to develop than in sib-crosses. Brink (1) has reported that only 15% of the ovules in alfalfa became fertile after selfing in contrast to 66% after cross-pollination. In the general analysis of variance (Table VI) a small advantage of out-crosses in the number of cells per embryo and of nuclei per endosperm was also highly significant but in more detailed direct comparisons the significance held only for nuclei in the endosperm. These results are remarkably similar to those obtained by Brink and Cooper (2) in alfalfa and discussed by Brink (1), who emphasized regarding the endosperm that "the decrease in size resulting from the inbreeding is so large that one is led immediately to suspect that herein lies the primary cause of the frequent seed collapse following selfing." In this connection it is pertinent to note that in our experiment I crosses, 23% of ovaries produced seed in sib-crosses and 38% in out-crosses and that the difference is statistically significant. Whatever the cause, our results indicate that outcrossing is advantageous.

It has been noted that embryo sacs are produced at about equal frequencies in the upper and lower ovules of both low- and high-seed-yielding plants. The percentages of these fertile ovules that start to develop are regularly higher in both upper and lower ovules of high-seed-yielding plants than of low-yielding ones. In most comparisons at 2 days the upper fertile ovules were developing more frequently than the lower ovules in both types of plants. The average difference between the percentages developing in upper and in lower ovules decreased to nearly zero by 4 days in high-seed-yielding plants, where 88% of the available ovules started to develop, but it decreased much less in the low-yielding ones where only 58% of the available ovules started to develop by 4 days. This again suggests that lower ovules are at a disadvantage because the pollen tubes grow more slowly in the styles and/or ovaries of low-seed-yielding plants. Cooper, Brink, and Albrecht (4) reported for alfalfa that "A greater proportion of the ovules at the stylar end of the ovary become fertile than at the base." They also recorded that "The higher percentages of fertile ovules in the basal region of the ovaries of the high seed-setting group may be explained by the fact that pollen tubes generally progress somewhat farther in the ovaries of these plants than they do in the ovaries of the low seed-setting individuals." If we accept that rate of growth of the pollen tubes may determine the extent of growth, there is no real difference in these interpretations.

As mentioned in the Introduction, a number of workers have described the collapse of ovules in legumes. Pandey (9) has described the process in red clover and his Table 2 seems to imply that it occurs as a rule in the lower ovule. In plants of experiment II collapse was started in 14% of developing upper ovules examined at 2 and 4 days and in 18% of lower developing ovules. At these early stages more ovules collapsed in high-seed-setting plants than in the low ones, probably because they possessed at that stage many more ovaries that contained two competing developing ovules. Between 4 days after pollination and maturity, ovule collapse was twice as frequent in low-seed-producing plants as in the high ones (60% vs. 32%, respectively, in experiment II). There was no indication, however, that the process of ovule collapse differed in these two types of plants. Not only did more ovules collapse in low-seed-yielding plants but also the resulting reduction of seed yield as a consequence was more extensive in them than in the high-seed-setting plants because most of their ovaries at these stages contain only one developing ovule whereas many ovaries of the high-seed-yielding plants have two developing ovules, one of which can be lost without a reduction in the expected yield of seed. Although the initial situation regarding ovule collapse differs from alfalfa in that collapse started earlier in our high-yielding plants than in the low ones, the end result regarding reduction in seed yield is very similar to that found by Cooper, Brink, and Albrecht (4) in alfalfa.

Using combined data from the four sets of comparable crosses (L × CL, L × CH, H × CL, and H × CH) of experiment I in 1954 and from tests 3–6 inclusive (L × CH, H × CH) of experiment II in 1955, the general features of development in high- and low-seed-yielding plants can be compared. For each 100 ovules (from 50 ovaries) of low-yielding plants we find that about 40 (40%) of them produce embryo sacs; of these fertile ovules only 23.2 (58%) start to develop by say 4 days; and of those that start only 5.6 (24%) avoid collapsing and mature into seed. These 5.6 ovules will be located in separate ovaries, so that 11.2% of the florets will produce a seed. The greatest numerical loss of ovules results from failure to produce embryo sacs but the proportionate loss from ovule collapse is of greater magnitude. In contrast, for each 100 ovules (from 50 ovaries) of high-yielding plants we find 72 (72%) of them produce embryo sacs, of these 61.9 (86%) start to develop by 4 days and 32.8 (53% of developing ovules) finally become seeds. The 32.8 seeds will occupy separate ovaries, so 65.8% of the florets will produce a seed. Here most losses of ovules result from failure to produce embryo sacs or from ovule collapse. Slow or limited growth of pollen tubes is apparently not very important when the female parent is high yielding but serious in the low-yielding ones, where it reduces the percentage of ovules fertilized and may increase the percentage of ovules that collapse. A plant that produces more fertile ovules will tend to lose numerically more through ovule collapse, which probably explains at least partly why it is difficult to get a high correlation between the percentages of fertile ovules and the percentages of florets setting seed.

These experiments indicate that there are three major causes of reduced seed yields in diploid red clover: failure to produce embryo sacs, slow or limited pollen tube growth, and the collapse of developing ovules. The failure to produce embryo sacs is frequent and therefore of special significance in red clover as compared with certain other leguminous plants. We have previously suggested (13) that genetic factors affect the ability of plants to produce embryo sacs. Slow or restricted growth of pollen tubes in low-seed-producing plants apparently delays fertilization and is followed by slow or delayed growth of embryo and endosperm, so that the number of ovules that collapse increases above and beyond the usual reduction to one seed per ovary. It looks as if the low-seed-yielding plants have certain genes that act like weak self-incompatibility alleles by inhibiting pollen tube growth with the consequent reduction in seed production through ovule collapse. On the other hand, it may be that the genetic constitutions of these low-seed-yielding plants are such that they cannot produce enough embryo sacs and/or support the growth of pollen tubes and ovules. These experiments do not provide a basis for choosing between these alternate interpretations.

Uniform categories of red clover plants regarding seed-yielding ability apparently do not exist. Each plant seems to have its own characteristic level of production of embryo sacs, of speed of fertilization, of success of fertilization, and of ovule collapse. It may be superior in any one of these features and inferior in any other, with all gradations between for each feature. Probably each environmental factor affects each of these features somewhat differently. The resulting interactions tend to confuse the interpretations of some statistical tests and considerations of seed yield in red clover.

Acknowledgments

During the course of these experiments we have received many helpful suggestions and valued co-operation from Professors H. A. Steppler and L. C. Raymond of the Department of Agronomy at Macdonald College, and we wish to acknowledge their assistance with sincere thanks and appreciation. The financial support of this study as EMR Project No. 57 by the Experimental Farms Service, Canada Department of Agriculture, Ottawa, is also acknowledged with due thanks. Thanks are also due to Professor G. I. Paul, who has advised us on some statistical problems.

References

1. BRINK, R. A. 1952. Inbreeding and crossbreeding in seed development. Heterosis, 81–97. Iowa State College Press.
2. BRINK, R. A. and COOPER, D. C. 1947. The endosperm in seed development. Botan. Rev. **13**, 423–541.
3. COOPER, D. C. and BRINK, R. A. 1940. Partial self-incompatibility and the collapse of fertile ovules as factors affecting seed formation in alfalfa. J. Agr. Research, **60**, 453–472.
4. COOPER, D. C., BRINK, R. A., and ALBRECHT, H. R. 1937. Embryo mortality in relation to seed formation in alfalfa (*Medicago sativa*). Am. J. Botany, **24**, 203–213.
5. DAS, B. CH. 1953. Cytological and embryological basis for sterility in autotetraploid sweetclover *Melilotus alba* Desr. Iowa State Coll. J. Sci. **27**, 537–561.

6. GREENSHIELDS, J. E. R. 1954. Embryology of interspecific crosses in *Melilotus*. Can. J. Botany, **32**, 447–465.
7. LEDINGHAM, G. F. 1940. Cytological and developmental studies of hybrids between *Medicago sativa* and a diploid form of *M. falcata*. Genetics, **25**, 1–15.
8. MARTIN, J. N. 1914. Comparative morphology of some Leguminosae. Botan. Gaz. **58**, 154–167.
9. PANDEY, K. K. 1955. Seed development in diploid, tetraploid and diploid–tetraploid crosses of *Trifolium pratense* L. Indian J. Genetics & Plant Breeding, **15**, 25–35.
10. PANDEY, K. K. 1956. Mutations of self-incompatibility alleles in *Trifolium pratense* and *T. repens*. Genetics, **41**, 327–343.
11. PANDEY, K. K. 1956. Incompatibility in *Trifolium pratense*. Genetics, **41**, 353–366.
12. POVILAITIS, B. and BOYES, J. W. 1956. A study of fertility in diploid Dollard red clover. Can. J. Agr. Sci. **36**, 59–71.
13. POVILAITIS, B. and BOYES, J. W. 1959. Embryo-sac production in relation to seed yields of diploid Dollard red clover. Can. J. Plant Sci. **39**, 364–374.
14. WILLIAMS, R. D. 1939. Incompatibility alleles in *Trifolium pratense* L. and their frequency and linkage relationships. Proc. Intern. Congr. Genet. 6th Congr. **7**, 316.
15. WILLIAMS, R. D. and SILOW, R. A. 1933. Genetics of red clover (*Trifolium pratense* L). Compatibility. I. J. Genet. **27**, 341–362.
16. WILLIAMS, R. D. and WILLIAMS, W. 1947. Genetics of red clover (*Trifolium pratense* L). Compatibility. III. The frequency of incompatibility S alleles in two non-pedigree populations of red clover. J. Genet. **48**, 69–70.
17. WHITE, C. 1952. The use of ranks in a test of significance for comparing of two treatments. Biometrics, **8**, 33–41.

POPULATION AND VIABILITY STUDIES OF HELMINTHOSPORIUM SATIVUM IN FIELD SOILS[1]

S. H. F. CHINN, R. J. LEDINGHAM, AND B. J. SALLANS

Abstract

The population and viability of spores of *Helminthosporium sativum* from two locations in each of 100 cultivated fields in Saskatchewan were determined in the spring of 1959. The determinations were made by the flotation–viability count method as described herein. Spore numbers in the different samples ranged from a count of less than 8 to 893 with an average of 118 per gram of soil. A predominant number of the samples contained 200 or less spores per gram. Soils summerfallowed or cropped to cereals in 1958 did not differ significantly in spore populations. The coefficient of correlation between the populations of the paired samples in the fields was 0.83. Viability of spores varied from 11 to 100%; a relatively small number of the samples was in the 11 to 30%, and the others were fairly evenly distributed in the 31 to 100% range. Evidence is presented to support the reliability of the flotation–viability count method. The applicability of the method to studies of various root rot problems and the behavior of certain fungi, other than *H. sativum*, in soil is discussed.

Introduction

The widespread occurrence of spores of *Helminthosporium sativum* Pamm., King, and Bakke (*Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur), main causative organism of common root rot of wheat, in prairie soil, is generally recognized, mainly because of root rot severity and the frequent isolation of the fungus from diseased plants. Since these manifestations of the disease are influenced by environmental conditions they may not be related quantitatively to the spore content of the soil.

Some attempts have been made to estimate more directly the occurrence of *H. sativum* in soil. Bisby, James, and Timonin (1), in their study of fungi from Manitoba by the plate method, found *H. sativum* in about 10% of the soil samples. Using essentially the same method in a study on the survival of microorganisms introduced into the soil, Katznelson (4) noted that changes in population over a period of time could be estimated with some but not all fungi. *H. sativum* was among those difficult to distinguish on fungal isolation plates. More recently Ledingham and Chinn (3) using a strictly quantitative method, designated as the flotation count, were able to show the presence of the fungus in all of the 20 soils sampled near Saskatoon.

This paper reports on the relative abundance of *H. sativum* spores in soil from 100 Saskatchewan farms. It was possible, with the method described herein, to estimate with accuracy not only the spore population but also the viability of the spores of this pathogen.

[1]Manuscript received March 22, 1960.
Contribution No. 40, Canada Department of Agriculture Research Station, Saskatoon, Sask.

Determination of Population and Viability of Spores of *H. sativum*

Flotation–Viability Count Method

The method used for determining spore population and viability in soil is designated as the flotation–viability count method. It is a modification of the flotation–viability test (2), by the incorporation of a counting technique. The method was as follows: the soil to be tested was screened and water added where necessary to bring the moisture content to a moderate level (approximately 40% of moisture holding capacity). Forty grams were thoroughly mixed with 10 ml of mineral oil in a Petri dish and then transferred to a 28×300 mm test tube. Tap water was added until the contents reached to within 6 cm of the top of the tube. The tube was stoppered, agitated vigorously for 3 to 5 minutes, and placed in a vertical position. In less than 15 minutes most of the soil had settled, leaving an emulsion containing the spores above the water. The emulsion, amounting to about 25 ml, was pipetted into a weighed 30×70 mm screw-cap jar and warmed to 45° C. Potato dextrose agar containing 1% molasses was melted and cooled to 45° C; then 8 ml were added to the emulsion. The jar and mixture were weighed. The mixture was shaken vigorously and a clean microscope slide was dipped momentarily into the agar suspension. The jar and contents were again weighed. As soon as the agar had set on the slide (about 1 minute), it was placed in a staining dish fitted with moist filter paper. A further precaution against drying of the agar on the slide was taken by placing the dish in a second closed container. The slide was incubated at 24° C for 15 to 20 hours.

At the end of the incubation period the agar on one side of the slide was wiped off and a dilute solution of cotton blue was sprayed on the opposite side with an atomizer. The entire surface of the agar suspension on the slide was examined microscopically at a magnification of about 80. Both germinated and non-viable spores of *H. sativum* were recorded.

Numerical estimates of spore populations, i.e., spores per gram of soil, were computed by multiplying the number of spores on one side of the slide by a specific factor (varying from 7 to 9). The factor designed to convert the spore population from a 40-g to a 1-g basis was derived as follows:

$$\text{factor} = \frac{\text{total weight of emulsion and agar}}{\text{weight of emulsion and agar on one side of a slide}} \times \frac{1}{40} .$$

Studies on Accuracy of Count

The accuracy of this method for estimating spore population and viability of *H. sativum* in soil was determined in a number of trials. Since all the trials gave similar conclusions only two will be described in some detail.

In one of the trials spore suspensions of *H. sativum* at rates to make up a geometric series were mixed with 2000-g lots of a natural soil that was entirely devoid of the pathogen. An uninfested lot was included. Determinations of spore population were made four times at 3-day intervals. The soil was stored at 2° C during the experiment.

TABLE I

Comparison of spore population of *H. sativum* as determined by the flotation–viability count method and the expected number as based on the total numbers of spores recovered or the total number of spores added

Units of spore suspensions	Replicates				Expected no. of spores based on:†	
	1	2	3	4		
	Observed no. of spores recovered from soil*				Number recovered	Number added
0	0	0	0	0	0	0
1	4	4	2	2	2.73	2.78
2	7	6	7	7	5.47	5.56
4	10	11	15	17	10.93	11.12
8	22	31	23	27	21.87	22.24
16	48	50	50	48	43.75	44.48
32	94	93	99	74	87.50	88.96
64	189	153	195	162	175.00	177.92
128	371	312‡	367	289‡	350.00	355.84
255	745	660	758	626	697.25	708.90

*In order to use the chi-square method for comparison, the observed number of spores on slides and not the spore population per gram of soil was used.
†Based on total number of spores added (697.25), the total number of spores recovered (708.90), and the geometric ratio of units of spore suspensions in the various lots of soil.
‡Number of spores recovered differ significantly from the expected according to χ^2 test.

Of the 36 spore counts recorded in Table I only two differed significantly by the χ^2 test from the expected values; therefore the counts are good estimates of the numbers of spores present in the soil. Increasing the number of determinations from one to two or more increased the reliability of the counts.

The percentage recovery of spores can be estimated from Table I by comparison with the number of spores added to the soil given in the last column, based on hemacytometer counts of spores in the original spore suspension. The total spores recovered in each replicate were 105, 93, 107, and 88%, averaging 98% of the spores added to the soil.

The variation in spore counts from the expected tended to be high when the counts from a slide were low and also when they were high. The best estimates were obtained when the number of spores varied from about 25 to 200 per slide.

In another trial the reliability of the method was indicated by the consistency of results. Five natural soils of widely differing types were collected, screened, and stored at 2° C. Four samples from each soil were examined for spore population and viability. Results are shown in Table II.

The χ^2 test indicates that only in the clay loam was there any significant departure in replicate counts from the mean count for each soil sample. With one exception determinations of viability of spores were quite consistent. In the case of the silty clay loam the percentages varied from 13 to 38%. Variations such as these can be expected to occur where the spore count on a slide is low. A difference of one viable or non-viable spore as in this specific instance accounted for about a 12% variation.

TABLE II

Replicate determinations of population and viability of spores of *H. sativum* in five natural soils by the flotation–viability count method

Soil type	No. of spores per slide*						Percentage viable spores				
	Repl. 1	Repl. 2	Repl. 3	Repl. 4	Mean	χ^2	Repl. 1	Repl. 2	Repl. 3	Repl. 4	Mean
Sandy loam	39	33	40	46	39.5	2.152	92	90	90	89	91
Loam	0	0	0	0	0	—	—	—	—	—	—
Silty clay loam	8	7	8	7	7.5	0.133	13	29	38	29	27
Clay loam	71	69	106	100	86.5	12.821	96	91	92	97	93
Heavy clay	24	27	21	22	23.5	0.894	88	96	86	90	90

*In order to use the chi-square method for comparison, the observed number of spores on slides and not the spore population per gram of soil was used.

Occurrence of Spores of *H. sativum* in Field Soil

Sampling Methods

Two soil samples from each of 100 farms located within a radius of 85 miles of Saskatoon were examined for population and viability of *H. sativum* spores by the flotation–viability count method. Samplings were made in the spring of 1959 at which time a small percentage of the fields had been seeded. There were 81 fallow and 19 other fields that were disked but had been cropped to wheat or barley the previous year. The latter group will be referred to as stubble fields. The samples from each field were taken 50 ft apart, one being 50 ft from the edge and the other further toward the center of the field.

Each sample, amounting to about 20 lb, was taken from the surface 2.5 to 3 in. of soil. The soils were put in plastic bags, brought to the laboratory, screened, and examined for populations and viability of *H. sativum* spores. When determinations could not be made immediately, the soils were stored at 2° C.

Results

The spore populations of the 200 soil samples are shown in Table III. The populations of the fallow and stubble fields are listed separately, the whole range of population being divided into classes. The distribution of samples into the various classes for stubble fields is generally similar to that for fallow fields. The mean number per gram for stubble fields, 148, exceeds that for fallow fields, 111, by 37. This, however, is not a significant difference because of the great variability in spore population between samples. The average number of spores for all samples was 118 per gram of soil. Five soil samples contained less than 8 spores (8 was the factor most commonly used for conversion of spore number per slide to spore number per gram of soil), 169 contained 8 to 200, 23 contained 201 to 500, and 3 contained 501 to 1000 spores per gram of soil. The curve of distribution of samples when divided into class ranges of 50 resembles a Poisson curve.

TABLE III

Distribution of 200 soil samples on basis of number of spores of *H. sativum* per gram of soil

Number of spores in samples	Numbers of soil samples		
	Fallow	Stubble	Total
Less than 8	5	0	5
8– 50	44	8	52
51– 100	55	12	67
101– 200	41	9	50
201– 300	9	3	12
301– 400	3	3	6
401– 500	2	3	5
501–1000	3	0	3
	162	38	200

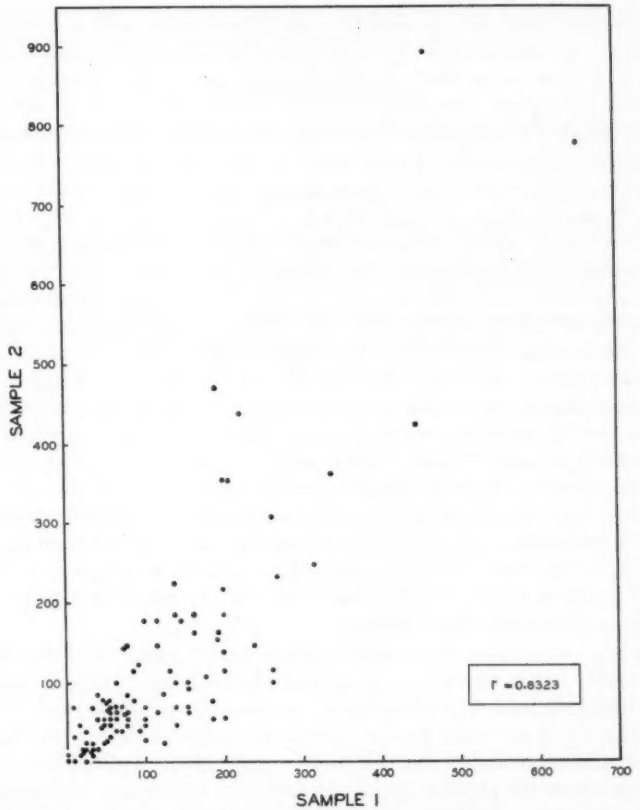


FIG. 1. Scatter diagram showing data on spore populations per gram of soil of *Helminthosporium sativum* and the correlation between paired soil samples from 100 fields.

The correlation between the spore population of the paired samples from the 100 fields is shown in Fig. 1. The coefficient of correlation is 0.83.

The percentages of spores that were viable in the 195 soil samples (spores were not observed on slides from 5 samples) were as follows:

Percentage of viable spores	No. of soil samples
0– 10	0
11– 20	5
21– 30	11
31– 40	22
41– 50	28
51– 60	19
61– 70	25
71– 80	35
81– 90	26
91–100	24

Discussion

The flotation–viability method of spore counts may have wide application in studies of *H. sativum* in soil. In addition to field sampling it is being used in conjunction with a number of projects that are being investigated at the Saskatoon laboratory. For instance, in ecological studies of *H. sativum*, soil is examined at various seasons or periods for fluctuations in spore population and viability. Information is also being obtained on the influence of cultural and cropping practices, chemical treatment, and natural conditions on the spores. Present findings of the existence of large differences in population and wide variation in the numbers of viable spores in both fallow and stubble fields require some explanation. Undoubtedly, a portion of the variability in the data resulted from experimental errors. However, the variation in spore numbers from field to field in either the fallow or stubble group could be expected since these fields were not cropped identically and environmental conditions differed. Although the coefficient of correlation (0.83) between the spore population of the paired samples from the 100 fields is statistically significant at the 1% level the scatter diagram (Fig. 1) indicates there is considerable variation in spore counts within fields. To reliably estimate spores in a field would involve adequate sampling techniques. Even with only two sample locations in each field, however, it would be possible to classify the fields on the basis of spore counts into three or four broad groups. The primary purpose of the counts in this work, however, was not to make close comparisons between fields so much as to get accurate counts on the samples of soil to be used in infection studies, to be reported later.

Other data suggesting the need for further investigation were also obtained in this study. In the Vanscoy area soil samples were taken from a fallow field and an adjoining field that was cropped to wheat the previous year. The spore population was 8 per gram for the former as compared to 281 for the latter. However, this relationship did not exist in a similar pair of fields in the Pike Lake area where the population was 44 and 56, respectively. Differences such as these may be explained through observations over a period of years on farming practices and environmental conditions. The flotation–viability count

method should greatly enhance present knowledge of the environmental conditions that influence spore population and viability in soil.

Often in soil inoculation studies excessive numbers of spores are added. The present study has indicated maximum and minimum numbers of spores of *H. sativum* that occur in soil under natural conditions. Thus it would seem useless in most of these studies to add spores in excess of populations reported here. Excessive numbers may even give results differing from those where more normal numbers were used.

Whether spore population or environmental factors play the more important role in the common root rot disease has received very little attention. Data obtained in conjunction with the present study will to some measure elucidate the relative importance of one or the other. This phase of the work is now under study.

In the examination of slides, spores of *Alternaria*, *Trichothecium*, and other fungi were also observed. This suggests the applicability of the method to the study of certain fungi, other than *H. sativum* in soil.

Acknowledgments

The authors wish to express their appreciation to Mrs. J. Key for valuable technical assistance.

References

1. Bisby, G. R., James, N., and Timonin, M. 1933. Fungi isolated from Manitoba soil by the plate method. Can. J. Research, **8**, 253–275.
2. Chinn, S. H. F. and Ledingham, R. J. 1958. Application of a new laboratory method for the determination of the survival of *Helminthosporium sativum* spores in soil. Can. J. Botany, **36**, 289–295.
3. Ledingham, R. J. and Chinn, S. H. F. 1955. A flotation method for obtaining spores of *Helminthosporium sativum* from soil. Can. J. Botany, **33**, 298–303.
4. Katznelson, H. 1940. Survival of microorganisms introduced into soil. Soil Sci. **49**, 283–293.

CHROMOSOME NUMBERS AND TAXONOMIC NOTES ON NORTHERN GRASSES

III. TWENTY-FIVE GENERA[1]

WRAY M. BOWDEN

Abstract

Chromosome numbers and voucher specimens are recorded for some collections of 25 genera of grasses, mainly collected in Canada. The somatic chromosome numbers of the following are recorded: (1) *Agrostis borealis*, $2n = 56$; *A. canina*, $2n = 14$; *A. gigantea*, $2n = 42$; *A. palustris*, $2n = 30$; *A. perennans*, $2n = 42$; *A. scabra*, $2n = 42$; and *A. tenuis*, $2n = 28$; 32; and 34. (2) *Alopecurus aequalis*, $2n = 14$; *A. alpinus*, $2n = 105$; 112; and 119; *A. geniculatus*, $2n = 28$; *A. occidentalis*, $2n = 112$; and *A. pratensis*, $2n = 28$. (3) *Ammophila breviligulata*, $2n = 28$. (4) *Anthoxanthum odoratum*, $2n = 20$. (5) *Calamagrostis canadensis*, $2n = 42$; and 56; *C. inexpansa*, $2n = 70$; *C. lapponica*, $2n = 28$; 32; 42; ca. 91; 98; and 140; *C. neglecta*, $2n = 28$; *C. pickeringii*, $2n = 28$; *C. purpurascens*, $2n = 28$; 42; and 84; and *Calamagrostis* sp.?, putative hybrid, $2n = 49$. (6) *Cinna arundinacea*, $2n = 28$; and *Cinna latifolia*, $2n = 28$. (7) *Danthonia compressa*, $2n = 36$; and *D. spicata*, $2n = 36$. (8) *Deschampsia brevifolia*, $2n = 26$; *D. caespitosa*, $2n = 26$; 27; and 28; *D. elongata*, $2n = 26$; *D. flexuosa*, $2n = 28$; and 32; and *D. pumila*, $2n =$ ca. 39. (9) *Hierochloë alpina*, $2n = 56$; and *H. odorata*, $2n = 56$. (10) *Koeleria cristata*, $2n = 14$. (11) *Leersia virginica*, $2n =$ ca. 48. (12) *Milium effusum*, $2n = 28$. (13) *Muhlenbergia squarrosa*, $2n = 40$; and *M. uniflora*, $2n =$ ca. 42. (14) *Nardus stricta*, $2n = 30$. (15) *Oryzopsis asperifolia*, $2n = 48$; *O. pungens*, $2n = 24$; and *O. racemosa*, $2n = 48$. (16) *Panicum xanthophysum*, $2n = 36$. (17) *Phalaris arundinacea*, $2n = 28$. (18) *Phleum alpinum*, $2n = 28$; and *Phleum pratense*, $2n = 42$. (19) *Sieglingia decumbens*, $2n = 36$. (20) *Sorghastrum nutans*, $2n = 40$. (21) *Spartina gracilis*, $2n = 42$. (22) *Sphenopholis intermedia*, $2n = 14$. (23) *Sporobolus cryptandrus*, $2n = 36$. (24) *Stipa comata*, $2n = 44$. (25) *Trisetum flavescens*, $2n = 28$; *T. molle*, $2n = 42$; and *T. spicatum*, $2n = 28$.

Introduction

Bowden (1959, 1960*a*) reported the chromosome numbers of some collections of the tribes TRITICEAE and FESTUCEAE. In the present paper, the third part of the series, collections of 25 genera are considered. These genera belong to several different tribes and since taxonomists have not yet agreed on a final classification of all of the grass tribes, the 25 genera are listed in alphabetical sequence for ready reference. Bowden (1959) described the materials and methods that have been used during these investigations.

Results

The species in each genus are arranged alphabetically so that the data can be easily consulted. For each voucher specimen, data are given on the province, district, or state, the cytogenetic accession number (= Cyt. No.), the geographical locality where the material was originally collected, the collector's name and specimen number, and the somatic ($2n$) chromosome number. In the citation of chromosome numbers, the specimens sent in from the field as

[1]Manuscript received April 5, 1960.
Contribution No. 71 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

live plants are designated as "clones" and the plants grown to maturity from seed are designated as "plants". All of the voucher specimens are deposited in the Phanerogamic Herbarium of the Plant Research Institute (DAO).

(1*a*) *Agrostis borealis* Hartm.

Plants of seven collections were octoploid, $2n = 56$.

Voucher specimens: Quebec: Cyt. No. 650; 651; 661; and 696; Fort Chimo, *J. A. Calder s.n., August, 1948 and J. A. Calder 2517*; $2n = 56$ det. on three clones and one plant. Cyt. No. 955, Great Whale River, *D. B. O. Savile 649*; $2n = 56$ det. on one plant. Labrador: Cyt. No. 1836, Saglek, *J. M. Gillett 9060A*; $2n = 56$ det. on one clone. New York: Cyt. No. 1590, Whiteface Mountain, *W. G. Dore s.n., June, 1955*; $2n = 56$ det. on one clone.

The octoploid chromosome number was reported for this species by Björkman (1951, 1954), Böcher and Larsen (1950), Jørgensen *et al.* (1958), and Sokolovskaya (1938).

(1*b*) *Agrostis canina* L. var. *canina*

Plants of four collections from Newfoundland were diploid, $2n = 14$.

Voucher specimens: Newfoundland: Cyt. No. 813; 814; 841; and 846; Gander, *I. J. Bassett s.n., July, 1949*; $2n = 14$ det. on four clones.

European material of *A. canina* var. *canina* was found to be diploid by Björkman (1951, 1954) and Jones (1956).

(1*ci*) *Agrostis gigantea* Roth var. *gigantea*

Two collections of the typical variety were hexaploid, $2n = 42$.

Voucher specimens: Ontario: Frontenac Co.: Cyt. No. 1509 and 1512, about 0.5 mile north of Collins Bay, near Kingston, *W. G. Dore 13814 and 13816*; $2n = 42$ det. on two clones.

Tischler (1950) listed numerous authors who reported the hexaploid number for this species. Björkman (1954) reported many hexaploid plants of *A. gigantea* and two plants had supernumeraries.

(1*cii*) *Agrostis gigantea* Roth var. *dispar* (Michx.) Philipson

Seven collections were hexaploid, $2n = 42$.

Dr. W. G. Dore examined these specimens and concluded that they are the same as *Agrostis alba* L. in Chase's revision of Hitchcock's manual (1951). However, the name *Agrostis alba* L. should not be used for this taxon. For *A. alba*, Linnaeus (1753) originally wrote "9. Agrostis panicula laxa, calycibus muticis aequalibus. *Roy. lugdb.* 59. *Habitat in* Europae *nemoribus.*" In the Phanerogamic Herbarium (DAO), there are photographs and a fragment of the type specimen of *A. alba* from the Van Royen Herbarium at Leiden (L) and the specimen is a *Poa*, probably *Poa nemoralis* L.

Voucher specimens of var. *dispar*: Newfoundland: Cyt. No. 830; 850; 855; 858; and 860; Gander, *I. J. Bassett s.n., July, 1949*; $2n = 42$ det. on five clones. Prince Edward Island: Cyt. No. 1559, $3\frac{1}{2}$ miles northwest of Charlottetown, *D. Erskine 1599*; $2n = 42$ det. on one plant. Ontario: Frontenac Co.: Cyt. No. 1511, about 0.5 mile north of Collins Bay, near Kingston, *W. G. Dore 13817*; $2n = 42$ det. on one clone.

(1*d*) *Agrostis palustris* Huds.

One collection was tetraploid with two extra chromosomes, i.e., $2n = 30$. The name *A. palustris* Huds. is here used in the sense of Hitchcock (1951).

Voucher specimen: Ontario: Frontenac Co.: Cyt. No. 1508, near Kingston, *W. G. Dore 13815*; $2n = 30$ det. on one clone.

Church (1936) reported that plants of *A. palustris* were tetraploid with $n = 14$.

(1*e*) *Agrostis perennans* (Walt.) Tuckerm.

One collection examined by the author was hexaploid, $2n = 42$, and another voucher specimen in DAO herbarium was reported as hexaploid by Björkman (1951).

Voucher specimens: Newfoundland: Cyt. No. 826, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 42$ det. on one clone. Ontario: District of Nipissing: Rankin Siding, 10 miles below Mattawa, *W. G. Dore & A. J. Breitung 9327*; $2n = 42$ det. on plants grown from seed from duplicate specimen; reported by Björkman (1951).

(1*f*) *Agrostis scabra* Willd.

Nine collections of this species examined by the author were hexaploid, $2n = 42$. Björkman (1951) reported another DAO specimen as hexaploid, and in a letter, Björkman (1952) listed another DAO specimen that he had determined as hexaploid.

Voucher specimens: Labrador: Cyt. No. 1061, Goose Bay, *J. M. Gillett s.n., July, 1950*; $2n = 42$ det. on one clone. Mackenzie District: Cyt. No. 1080, Salt River, *W. J. Cody & C. C. Loan 4602*; $2n = 42$ det. on one clone. Cyt. No. 894, Yellowknife, *W. J. Cody & J. B. McCanse 3027*; $2n = 42$ det. on one clone. Cyt. No. 918, Yellowknife, *W. J. Cody & J. B. McCanse 3242*; $2n = 42$ det. on one plant. Cyt. No. 1633, Norman Wells, in heavy soil of bank overlooking Mackenzie River, *W. J. Cody & R. L. Gutteridge 7497*; $2n = 42$ det. on one clone. Cyt. No. 1481, Lower Hay River, *W. H. Lewis s.n., August 14, 1951*; $2n = 42$ det. on one plant. Cyt. No. 1482, Lower Hay River, *W. H. Lewis 1109*; $2n = 42$ det. on one plant. Alaska: Cyt. No. 1366, Snow River valley north of Seward, Kenai Peninsula, *J. A. Calder 6561*; $2n = 42$ det. on one plant. Ontario: Cyt. No. 1510, Frontenac Co.: about 8 miles south of Kaladar, *W. G. Dore 13848*; $2n = 42$ det. on one clone. Björkman (1951) listed one accession of *A. hiemalis* (Walt.) B.S.P. as hexaploid, $2n = 42$. In a letter, Björkman (1952) corrected the identification of the specimen and stated that it should have been *A. scabra* Willd. He listed an additional accession which also proved to be hexaploid (*Dore & Breitung 9730*). These two specimens are: Ontario: Renfrew Co.: Mackey's Station, Head Twp., *W. G. Dore & A. J. Breitung 9730*; $2n = 42$ det. on plants grown from seed from duplicate specimen by Björkman (1952). Thunder Bay District: Terrace Bay, near Schreiber, *W. G. Dore 9239*; $2n = 42$ det. on plants grown from seed from duplicate specimen by Björkman (1951), listed under *Agrostis hiemalis*.

(1*g*) *Agrostis tenuis* Sibth.

Eight collections were tetraploid and some had extra chromosomes, i.e. $2n = 28$; $2n = 32$; and $2n = 34$.

Voucher specimens: Newfoundland: Cyt. No. 819 and 856, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 28$ det. on two clones. Cyt. No. 870, St. John's, *I. J. Bassett s.n., July, 1949*; $2n = 28$ det. on one clone. Cyt. No. 835, 839, and 852, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 32$ det. on three clones. Cyt. No. 821 and 849, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 34$ det. on two clones.

The somatic chromosome number $2n = 28$ has been reported for *Agrostis tenuis* by many authors. These literature reports have been summarized by Löve and Löve (1956). Stuckey and Banfield (1946) examined *Agrostis* plants from Rhode Island and adjacent states and, for some plants of *A. tenuis*, they reported $2n = 28$ and for a much larger number of plants, they observed counts from $2n = 29$ to $2n = 41$. Björkman (1954) examined large numbers of plants and reported that they were mostly tetraploid and a few plants were trisomics or had small supernumeraries.

(2*a*) *Alopecurus aequalis* Sobol.

Sixteen collections of this species were uniformly diploid, $2n = 14$. Nine accessions were *A. aequalis* sensu str. ("*aristis gluma aequalibus*"); one accession was a mixture of typical specimens and specimens with intermediate-length awns on the lemmas (Cyt. No. 1448); and six collections had much longer lemma awns (Cyt. Nos. 1486; 1427; 1424 and 1425 (from *Dore & Breitung 12,548 A and B*); 1422; and 1426. Dr. W. G. Dore (unpublished) has examined these specimens and has noted that the long-awned specimens are frequently confused with *Alopecurus geniculatus* L. which is always tetraploid, $2n = 28$. Moss (1959) listed *A. geniculatus* in the flora of Alberta but these plants are undoubtedly long-awned variants of *A. aequalis* similar to the six collections noted above. Lapshin (1939) listed four varieties of *Alopecurus aequalis* and *A. aequalis* Sobol. var. *sibiricus* Kryl. was described as: ["with an awn 1.7–2.5 mm. long, exserted from the spikelets for 0.75–1.3 mm."]. Whether or not the long-awned specimens from Alberta and Saskatchewan are identical with var. *sibiricus* is a problem that I shall leave for Dr. Dore to elucidate.

Voucher specimens: Prince Edward Island: Cyt. No. 1556, Prince Co.: Wellington, *D. Erskine 1410*; $2n = 14$ det. on one plant. Ontario: Cyt. No. 1833, Carleton Co.: Mer bleue, south of Blackburn Station, *W. G. Dore & W. M. Bowden 15356*; $2n = 14$ det. on one clone. Cyt. No. 1867, Durham Co.: 10 miles southwest of Peterborough, *W. M. Bowden 157-55*; $2n = 14$ det. on one clone. Mackenzie District: Cyt. No. 912, Yellowknife, Latham Island, *W. J. Cody & J. B. McCanse 3085*; $2n = 14$ det. on one plant. Cyt. No. 922, Indin Lake, *W. J. Cody & J. B. McCanse 3461*; $2n = 14$ det. on one plant. Yukon: Cyt. No. 1454, Porcupine River, near Rampart House on the Yukon–Alaska border, *J. E. H. Martin 105*; $2n = 14$ det. on one plant. Alaska: Cyt.

No. 1367, about 2–3 miles north of Seward, Kenai Peninsula, *J. A. Calder 6618*; $2n = 14$ det. on one plant. Cyt. No. 1401, Birch Lake, Mile 309, Richardson Highway, *W. J. Cody & T. J. M. Webster 5909*; $2n = 14$ det. on one plant. North Dakota: Cyt. No. 1420, Bottineau Co.: 5 miles west of Westhope, *W. G. Dore & B. Boivin 13,358*; $2n = 14$ det. on one plant. Saskatchewan: Cyt. No. 1448, Trossachs, 15 miles west of Weyburn, *W. G. Dore & B. Boivin 13,247*; $2n = 14$ det. on one plant. Cyt. No. 1422, Trossachs, *W. G. Dore & B. Boivin 13,240;* $2n = 14$ det. on one plant. Cyt. No. 1424 and 1425, 5 miles southeast of Weyburn, *W. G. Dore & A. J. Breitung 12,548 A and B*; $2n = 14$ det. on two plants. Alberta: Cyt. No. 1486, 10 miles northwest of Redcliffe, *E. H. Moss 9612*; $2n = 14$ det. on one plant. Cyt. No. 1427, western edge of Milk River Ridge, 12 miles east of Cardston, *W. G. Dore 12380*; $2n = 14$ det. on one plant. Cyt. No. 1426, north edge of Milk River Ridge, 40 miles south of Lethbridge, *W. G. Dore 11712*; $2n = 14$ det. on one plant.

Alopecurus aequalis is diploid, $2n = 14$, throughout its range. Löve and Löve (1956) listed numerous authors who reported the diploid chromosome number for this species. Jørgensen *et al.* (1958) also reported the same number.

(2*b*) *Alopecurus alpinus* J. E. Smith

Four collections were examined cytologically and three polyploid numbers were observed: $2n = 105$; $2n = 112$; and $2n = 119$. Under the related *A. occidentalis*, another $2n = 112$ collection is listed below. These and other related high polyploids probably belong in one species complex with a number of subspecies and varieties.

Voucher specimens of *A. alpinus*: Franklin District: Cyt. No. 611, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n = 105$ det. on one clone. Cyt. No. 582, Baffin Island, Frobisher Bay, *H. A. Senn s.n., July, 1948*; $2n = 112$ det. on one clone. Cyt. No. 607, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n = 119$ det. on one clone. Keewatin District: Cyt. No. 698, Southampton Island, Coral Harbour, Munn Bay, *W. J. Cody 1732*; $2n = 119$ det. on one plant.

Flovik (1940) examined material of *A. alpinus* from Spitzbergen and reported $2n = 112 + 3$ ff.; $2n = 114 + 2$ ff.; and $2n = 130 + 1$ f. Johnsson (1941) observed $2n = 119$ to $2n = 122$ in material of *A. alpinus* and $2n = 112$ to $2n = 116$ in plants of *A. antarcticus*. Holmen (1952) reported $n =$ ca. 56 in material of *A. alpinus* from Greenland and Jørgensen *et al.* (1958) reported $2n =$ ca. 112 and $n =$ ca. 56 in plants from Greenland.

(2*c*) *Alopecurus geniculatus* L.

Two accessions of this species were tetraploid, $2n = 28$.

Voucher specimens: Newfoundland: Cyt. No. 1405, St. Anthony, *D. B. O. Savile & J. Vaillancourt 2562*; $2n = 28$ det. on one plant. Nova Scotia: Cyt. No. 1485, Richmond Co.,West L'Ardois, *E. C. Smith et al. 5154*; $2n = 28$ det. on two plants.

Numerous cytologists have reported that this species is tetraploid, $2n = 28$, and Tischler (1950) has summarized the earlier literature. Löve and Löve

(1956) reported the tetraploid number for material of *A. geniculatus* from Iceland. Bosemark (1957) found $2n = 28$ in 15 plants and Johnsson (1941) observed the same chromosome number in 8 plants of this species.

(2*d*) *Alopecurus occidentalis* Scribn. & Tweedy

One collection of this species was a high polyploid with $2n = 112$.

Voucher specimen: Alberta: Cyt. No. 1445, 6 miles northwest of entrance to Waterton Lakes National Park, *W. G. Dore 12164*; $2n = 112$ det. on one plant.

Hultén (1942) listed specimens similar to this collection under *A. glaucus* Less. Sokolovskaya and Strelkova (1938) reported $2n = 130$ for *A. glaucus*.

(2*e*) *Alopecurus pratensis* L.

Three accessions were examined cytologically and the plants were tetraploid, $2n = 28$.

Voucher specimens: Newfoundland: Cyt. No. 781; 782; and 783; near Stephenville, *I. J. Bassett s.n., June, 1949*; $2n = 28$ det. on three clones. Cyt. No. 782 was determined by Dr. W. G. Dore as f. *breviaristatus* Beck and the other two collections as the typical form.

Tischler (1950) listed a large number of authors who have recorded $2n = 28$ for this species. Löve and Löve (1956) observed $2n = 28$ in Icelandic material of *A. pratensis* and Bosemark (1957) reported $2n = 28$ with 0–6 accessory chromosomes. Johnsson (1941) found one individual with $2n = 42$.

(3) *Ammophila breviligulata* Fern.

Three collections of this species were tetraploid, $2n = 28$.

Voucher specimens: Quebec: Cyt. No. 1704, Argenteuil Co., 1 mile east of Lachute; introduced from Prince Edward Island; *W. M. Bowden & B. Boivin 70-53*; $2n = 28$ det. on one clone. The introduction of this material is recorded in the reports of the Entomologist and Botanist, Experimental Farms Reports, for 1893–1904. Ontario: Cyt. No. 1688, Algoma, Pancake Bay, north of Sault Ste. Marie, *W. M. Bowden 42-53*; $2n = 28$ det. on one clone. New York: Cyt. No. 1597, Clinton Co., Cumberland Bay, Plattsburg, *W. G. Dore & J. M. Gillett 14311*; $2n = 28$ det. on one clone.

Church (1929) examined material of this species collected near Boston and reported $n = 14$.

(4) *Anthoxanthum odoratum* L.

Five accessions of *A. odoratum* were diploid, $2n = 20$.

Voucher specimens: Newfoundland: Cyt. No. 778; 779; 780; 784; and 785; near Stephenville, *I. J. Bassett s.n., June, 1949*; $2n = 20$ det. on five clones.

Löve and Löve (1956) reported $2n = 20$ for Icelandic material of *A. odoratum* and listed many references all of which listed the same chromosome number. Bosemark (1957) and Tateoka (1954) reported $2n = 20$ for material of this species and Hedberg (1958) reported the same chromosome number for specimens from Scotland.

(5*a*) *Calamagrostis canadensis* (Michx.) Nutt.

Numerous collections of this species were examined cytologically. Fifteen collections were hexaploid, $2n = 42$, and 12 accessions were octoploid, $2n = 56$. There are some morphological differences but it was impossible to clearly delimit the infraspecific taxa so that the specimens are listed under their respective chromosome numbers and no attempt is made to give an infraspecific classification.

Voucher specimens of the hexaploids: Newfoundland: Cyt. No. 812; 817; 818; 820; 825; 836; 838; 851; 859; Gander, *I. J. Bassett s.n., July, 1949*; $2n = 42$ det. on nine clones. Cyt. No. 873, St. John's, *I. J. Bassett s.n., July, 1949*; $2n = 42$ det. on one clone. Quebec: Cyt. No. 669, Fort Chimo, *J. A. Calder s.n., August, 1948*; $2n = 42$ det. on one clone. Mackenzie District: Cyt. No. 886, Yellowknife, *W. J. Cody & J. B. McCanse 3029*; $2n = 42$ det. on one clone. Cyt. No. 1617, Norman Wells, *W. J. Cody & R. L. Gutteridge 7506*; $2n = 42$ det. on one clone. Cyt. No. 1701, Norman Wells, *W. J. Cody & R. L. Gutteridge 7981*; $2n = 42$ det. on one clone. Cyt. No. 1877, Fort Simpson, *W. J. Cody & J. M. Matte 8780*; $2n = 42$ det. on one clone.

Voucher specimens of the octoploids: Newfoundland: Cyt. No. 843 and 861, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 56$ det. on two clones. Labrador: Cyt. No. 1069, Goose Bay, *J. M. Gillett s.n., July, 1950*; $2n = 56$ det. on one clone. Ontario: Cyt. No. 1503 and 1504, Frontenac Co., 7 miles NW. of Sharbot Lake, *W. G. Dore 13788 and 13787*; $2n = 56$ det. on two clones. Cyt. No. 1685, Norfolk Co., Turkey Point, *W. M. Bowden 50-53*; $2n = 56$ det. on roots fixed in field. Cyt. No. 1852, Carleton Co., between Harwood Plains and Shirley Bay, *W. M. Bowden 121-55*; $2n = 56$ det. on one clone. Manitoba: Cyt. No. 1119, Gillam, *W. B. Schofield 1497*; $2n = 56$ det. on one plant. Mackenzie District: Cyt. No. 882, Yellowknife, *W. J. Cody & J. B. McCanse 3039*; $2n = 56$ det. on one clone. Cyt. No. 1081, Salt River, *W. J. Cody & C. C. Loan 4592*; $2n = 56$ det. on one clone. Cyt. No. 1255, Lower Hay River, *W. H. Lewis 9*; $2n = 56$ det. on one clone. Cyt. No. 1878, Fort Simpson, *W. J. Cody & J. M. Matte 8784*; $2n = 56$ det. on one clone.

Böcher and Larsen (1950) reported $2n = 56$ for *C. canadensis* ssp. *langsdorfii*. Jørgensen *et al.* (1958) reported $2n = 51$ and $2n = 52$ for plants from Greenland (var. *scabra*) and cited counts of $2n = 56$ by Stebbins on material from the western U.S.A. Nygren (1954) examined collections from some of the eastern and western states of the U.S.A. and several locations in Canada and reported $2n = 42$; 45; 48; 49; 51; 56; 62; 65; and 66.

(5*b*) *Calamagrostis inexpansa* A. Gray

Two collections were examined cytologically and the plants were ten-ploid, $2n = 70$. Dr. W. G. Dore examined the specimens and classified them as var. *brevior* (Vasey) Stebbins.

Voucher specimens: Mackenzie District: Cyt. No. 1075, Salt Plain west of Fort Smith, *W. J. Cody & C. C. Loan 4569*; $2n = 70$ det. on one clone. Cyt. No. 1083, Salt Plain west of Fort Smith, *W. J. Cody & C. C. Loan 4582*; $2n = 70$ det. on one clone.

Nygren (1954) examined collections of *C. inexpansa* from California, Minnesota, and Colorado and reported $2n = 28$; 56; 58; 84; 85; 88; 89; 92; 98; 102; 103; and 105.

(5*c*) *Calamagrostis lapponica* (Wahl.) Hartm.

Seven collections of this species were examined cytologically and a series of polyploid chromosome numbers were observed: $2n = 28$; 32; 42; ca. 91; 98; and 140.

Voucher specimens: Manitoba: Cyt. No. 570, Fort Churchill, *J. M. Gillett s.n., July, 1948*; $2n = 28$ det. on one clone. Cyt. No. 569, Fort Churchill, *J. M. Gillett s.n., July, 1948*; $2n = 32$ det. on one clone. Quebec: Cyt. No. 653, Fort Chimo, *J. A. Calder, August, 1948*; $2n = 42$ det. on one clone. Mackenzie District: Cyt. No. 1746, Norman Wells, *W. J. Cody & R. L. Gutteridge 7827*; $2n =$ ca. 91 det. on one plant. Alaska: Cyt. No. 1276, near base of Donnelly Dome, Mile 250, Richardson Highway, *W. J. Cody 6297*; $2n = 98$ det. on one plant. Mackenzie District: Cyt. No. 895, Yellowknife, *W. J. Cody & J. B. McCanse 3025*; $2n =$ ca. 140 det. on one clone. Manitoba: Cyt. No. 555, Fort Churchill, *J. M. Gillett s.n., July, 1948*; $2n = 140$.

Nygren (1946) reported $2n = 42$; 49; ca. 91; ca. 98; ca. 105; and ca. 112 on a series of plants of *C. lapponica* from Sweden and observed much morphological variation. Böcher and Larsen (1950) reported $2n = 42$ for a collection of *C. lapponica* var. *groenlandica*.

(5*d*) *Calamagrostis neglecta* (Ehrh.) G.M.S.

Two collections of the species proved to be tetraploid, $2n = 28$.

Voucher specimens: Labrador: Cyt. No. 1064, Goose Bay, *J. M. Gillett s.n., July, 1950*; $2n = 28$ det. on one clone. Mackenzie District: Cyt. No. 880, Yellowknife, *W. J. Cody & J. B. McCanse 3038*; $2n = 28$ det. on one clone.

Löve and Löve (1956) reported $2n = 28$ for this species and summarized numerous literature reports of the same number (under two subspecies). Jørgensen *et al.* (1958) listed a tetraploid collection from Greenland.

(5*e*) *Calamagrostis pickeringii* A. Gray

One collection of this species was tetraploid, $2n = 28$.

Voucher specimen: Newfoundland: Cyt. No. 834, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 28$ det. on one clone.

(5*f*) *Calamagrostis purpurascens* R.Br.

Seven collections of *C. purpurascens* were examined; and four collections were tetraploid, $2n = 28$; two collections were hexaploid, $2n = 42$; and one collection was twelve-ploid, $2n = 84$.

Voucher specimens: Mackenzie District: Cyt. No. 1055, Fort Smith, *W. J. Cody & C. C. Loan 3932*; $2n = 28$ det. on one clone. Yukon: Cyt. No. 762; 764; and 773; Whitehorse, *J. M. Gillett s.n., June, 1949*; $2n = 28$ det. on three clones. Mackenzie District: Cyt. No. 883, Yellowknife, *W. J. Cody & J. B. McCanse 3034*; $2n = 42$ det. on one clone. Cyt. No. 915, Yellowknife, *W. J.*

Cody & J. B. McCanse 3238; $2n = 42$ det. on one plant. Alaska: Cyt. No. 1265, Big Delta Camp area, Mile 264, Richardson Highway, *W. J. Cody 6268*; $2n = 84$ det. on one plant.

Böcher and Larsen (1950) reported $2n = 56$ for a collection of this species from West Greenland. Nygren (1954) examined plants from California, Colorado, and one locality in Canada and reported $2n = 40$; 41; 42; 47; 48; 49; 50; 53; 54–57; and 56. Jørgensen *et al.* (1958) observed $2n = 58$ in a collection from northwest Greenland.

(5*g*) *Calamagrostis* sp.?, putative hybrid

One collection was seven-ploid, $2n = 49$, and it appears to be a hybrid between an octoploid, $2n = 56$, and a hexaploid, $2n = 42$.

Voucher specimen: Mackenzie District: Cyt. No. 1694, Cabin Creek, Mile 22 E, Canol Highway, *W. J. Cody & R. L. Gutteridge 7961*; $2n = 49$ det. on one clone.

(6*a*) *Cinna arundinacea* L.

Two collections were tetraploid, $2n = 28$.

Voucher specimens: Ontario: Cyt. No. 1825, Norfolk Co., Charlotteville Twp., Lot 10, Concn. II, *W. M. Bowden 87-54*; $2n = 28$ det. on one clone. Cyt. No. 1827, Grenville Co., northeast of Prescott, *W. G. Dore 15343*; $2n = 28$ det. on one clone.

(6*b*) *Cinna latifolia* (Trev.) Griseb.

One collection proved to be tetraploid, $2n = 28$.

Voucher specimen: Newfoundland: Cyt. No. 823, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 28$ det. on one clone.

Ehrenberg (1945) and Tateoka (1954) reported $2n = 28$ for this species.

(7*a*) *Danthonia compressa* Austin

One accession was hexaploid, $2n = 36$.

Voucher specimen: Quebec: Cyt. No. 1169, Gatineau Co., Farmer Rapids, 5 miles north of Ottawa, *W. G. Dore 12912*; $2n = 36$ det. on one plant.

De Wet (1954) listed $2n = 36$ for *D. compressa*.

(7*b*) *Danthonia spicata* (L.) Beauv.

Ten collections were hexaploid, $2n = 36$.

Voucher specimens: Ontario: Cyt. No. 1159, Durham Co., 2 miles west of Pontypool, *W. G. Dore 8691*; $2n = 36$ det. on one plant. Cyt. No. 1160, Essex Co., 5 miles SE. of Amherstburg, *W. G. Dore 8752*; $2n = 36$ det. on one plant. Cyt. No. 1162, Rainy River District, 10 miles north of Rainy River, *W. G. Dore 9144*; $2n = 36$ det. on one plant. Cyt. No. 1163, Brant Co., Spottiswood Lake, 10 miles south of Galt, *W. G. Dore 8711*; $2n = 36$ det. on one plant. Cyt. No. 1166, Brant Co., Spottiswood Lake, *W. G. Dore 8713*; $2n = 36$ det. on one plant. Cyt. No. 1164, Kenora District, Nestor Falls, *W. G. Dore 9096*; $2n = 36$ det. on one plant. Cyt. No. 1165, Bruce Co., South Sauble Beach, *W. G. Dore 8976*; $2n = 36$ det. on one plant. Cyt. No. 1168, Bruce Co., Southampton, *W. G. Dore 8934*; $2n = 36$ det. on one plant. Cyt. No. 1167,

Algoma District, 3 miles west of Thessalon, *H. A. Senn & D. R. Lindsay 5213*; $2n = 36$ det. on one plant. Cyt. No. 1457, Muskoka District, Camp Billie Bear, *J. H. Soper A.1*, $2n = 36$ det. on one plant.

De Wet (1954) listed $2n = 36$ for *D. spicata*.

(8*a*) *Deschampsia brevifolia* R.Br.

One accession was diploid, $2n = 26$.

Voucher specimen: Keewatin District: Cyt. No. 1531, Southampton Island, Coral Harbour, *D. K. Brown s.n., September, 1952*; $2n = 26$ det. on one clone.

Holmen (1952) reported $n = 13$ and $2n = 26$ for material of this species collected in North Greenland. Jørgensen *et al.* (1958) reported $n = 13$ for specimens collected in Greenland.

(8*b*) *Deschampsia caespitosa* (L.) Beauv.

Ten collections were examined cytologically and the somatic chromosome numbers $2n = 26$, $2n = 27$, and $2n = 28$ were observed.

Voucher specimens: Quebec: Cyt. No. 1894, Cap Rouge, *J. M. Gillett s.n., July, 1956*; $2n = 26$ det. on one clone. Ontario: Cyt. No. 1813, Carleton Co., about 2 miles above Billing's bridge, *W. G. Dore 13600*; $2n = 26$ det. on one clone. Cyt. No. 1814, Carleton Co., Shirley Bay, south shore of Ottawa River, *W. M. Bowden 84-54*; $2n = 26$ det. on one clone. Mackenzie District: Cyt. No. 1254, Lower Hay River, *W. H. Lewis 8*; $2n = 26$ det. on one clone. Cyt. No. 1622, Norman Wells, *W. J. Cody & R. L. Gutteridge 7494*; $2n = 26$ det. on one clone. Cyt. No. 1623, Norman Wells, *W. J. Cody & R. L. Gutteridge 7507*; $2n = 26$ det. on one clone. Cyt. No. 1631, Norman Wells, *W. J. Cody & R. L. Gutteridge 7504*; $2n = 26$ det. on one clone. Cyt. No. 1748, Norman Wells, *W. J. Cody & R. L. Gutteridge 7820*; $2n = 26$ det. on one plant. Cyt. No. 1749, Norman Wells, *W. J. Cody & R. L. Gutteridge 7819*; $2n = 26$ det. on one plant and $2n = 27$ det. on another plant. Cyt. No. 1076, Salt Plain west of Fort Smith, *W. J. Cody & C. C. Loan 4568*; $2n = 28$ det. on one clone.

Löve and Löve (1956) listed references for both $2n = 26$ and $2n = 28$ for this species. Hedberg (1958) reported $2n = 26$, 39, and 52 for collections of *D. caespitosa* (L.) P.B., s. lat., from Scotland.

(8*c*) *Deschampsia elongata* (Hook.) Munro

One collection was diploid, $2n = 26$.

Voucher specimen: British Columbia: Cyt. No. 1346, 4 miles west of Yahk, Highway No. 3, *H. A. Senn & C. Frankton 5872*; $2n = 26$ det. on one plant. Myers (1947) cited an unpublished determination of Stebbins as $2n = 26$ for this species.

(8*d*) *Deschampsia flexuosa* (L.) Trin.

Two collections were tetraploid, $2n = 28$ and $2n = 32$.

Voucher specimens: Ontario: Cyt. No. 1844, Carleton Co., Buckham Bay, near Constance Bay on the Ottawa River, *W. M. Bowden & L. Jenkins 113-55*; $2n = 28$ det. on one clone. Cyt. No. 1687; 1721, Algoma District, Pancake Bay, north of Sault Ste. Marie, *W. M. Bowden 44-53*; $2n = 28$ det. on one clone and three plants and $2n = 32$ det. on one plant.

The tetraploid number $2n = 28$ has been listed by numerous authors, for example, Jørgensen *et al.* (1958), Löve and Löve (1956, under *Avenella flexuosa*), Tateoka (1954), and Böcher and Larsen (1950).

(8*e*) *Deschampsia pumila* (Ledeb.) Ost.

One collection of this species was examined cytologically and proved to have a somatic count of $2n =$ ca. 39.

Voucher specimen: Franklin District: Cyt. No. 610, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n =$ ca. 39 det. on one clone.

Böcher and Larsen (1950) reported $2n = 39$ for material of this species from West Greenland and the problem of variability of this complex was discussed by Jørgensen *et al.* (1958).

(9*a*) *Hierochloë alpina* (Sw.) R. & S.

Three collections were examined cytologically and the plants were octoploid, $2n = 56$.

Voucher specimens: Franklin District: Cyt. No. 584, Baffin Island, Frobisher Bay, *H. A. Senn s.n., July, 1948*; $2n = 56$ det. on one clone. Keewatin District: Cyt. No. 1528, Southampton Island, Coral Harbour, *D. K. Brown s.n., September, 1952*; $2n = 56$ det. on one clone. New York: Cyt. No. 1589, Essex Co., near summit of Whiteface Mountain, elevation 4870 ft, *W. G. Dore s.n., June, 1953*; near *Dore 14270*; $2n = 56$ det. on one clone.

Jørgensen *et al.* (1958) observed $2n = 56$ in several collections of *H. alpina* from Greenland. Tateoka (1954) reported the same chromosome number for material of this species from Mt. Senjio. Flovik (1940) also reported the octoploid chromosome number for *H. alpina*.

(9*b*) *Hierochloë odorata* (L.) Beauv.

Five collections were octoploid, $2n = 56$.

Voucher specimens: Ontario: Cyt. No. 1579, Carleton Co., about 300 yards south of Beattie Point, south shore of Lake Deschenes, Ottawa River, *R. Roger & W. M. Bowden 14-53*; $2n = 56$ det. on two clones. Cyt. No. 1580, Carleton Co., 2 miles north of Manotick Station, Lot 24, Concn. III, *W. M. Bowden et al. 15-53*; $2n = 56$ det. on two clones. Cyt. No. 1582, Renfrew Co., 2.2 miles northwest of Chalk River, *W. M. Bowden, H. A. Senn, & J. M. Gillett 18-53*; $2n = 56$ det. on one clone. Saskatchewan: Cyt. No. 1822, North Battleford District, 10 milles à l'ouest de Frenchman Butte, *B. Boivin & D. Dunbar 10499*; $2n = 56$ det. on one clone. Mackenzie District: Cyt. No. 1739, Cabin Creek, Mile 22, Canol Highway, *W. J. Cody & R. L. Gutteridge 7942*; $2n = 56$ det. on one plant.

In view of the uniformly octoploid counts on the five collections of *H. odorata* listed above, it is difficult to interpret the different reports for this species in the literature. Church in Myers (1947) listed both $2n = 28$ and $2n = 56$ for *H. odorata*. Löve and Löve (1956) summarized the literature and listed both $2n = 28$ and $2n = 42$ for *H. odorata*; they stated that "this species is found to possess $2n = 28$ chromosomes in Iceland as well as in Sweden, Germany, and in North America (at least west to Manitoba)".

(10) *Koeleria cristata* (L.) Pers.

One collection of this species was diploid, $2n = 14$.

Voucher specimen: Ontario: Cyt. No. 1456, Lambton Co., about 4 miles NE. of Port Frank, *J. H. Soper & J. K. Shields 5039*; $2n = 14$ det. on one plant.

Tateoka (1955) reported $2n = 15$ and 16 and Stebbins and Love (1941) and de Wet (1960) listed $2n = 28$ for this species.

(11) *Leersia virginica* Willd.

One collection had a somatic number of $2n =$ ca. 48.

Voucher specimen: Quebec: Cyt. No. 1883, on banks of Leamy River, Hull, *W. M. Bowden & L. Jenkins 162-55*; $2n =$ ca. 48 det. on one clone.

Brown (1948) reported $n = 24$ for this species.

(12) *Milium effusum* L.

One collection was tetraploid, $2n = 28$.

Voucher specimen: Ontario: Cyt. No. 1848, Russell Co., near Navan, 13 miles E. of Ottawa, *W. G. Dore & L. Jenkins 15866*; $2n = 28$ det. on one plant. The specimen was identified as var. *cisatlanticum* Fern. by Dr. W. G. Dore.

Tateoka (1954) and Löve and Löve (1956) reported $2n = 28$ for this species.

(13*a*) *Muhlenbergia squarrosa* (Trin.) Rydb.

One collection was tetraploid, $2n = 40$.

Voucher specimen: Mackenzie District: Cyt. No. 1086, Salt Plain west of Fort Smith, *W. J. Cody & C. C. Loan 4581*; $2n = 40$ det. on one clone.

Stebbins and Love (1941) reported $2n = 40$ for *M. squarrosa* (listed under *M. richardsonis* (Trin.) Rydb.).

(13*b*) *Muhlenbergia uniflora* (Muhl.) Fern.

One collection had the somatic number $2n =$ ca. 42.

Voucher specimen: Quebec: Cyt. No. 1864, bog near small bridge on Gatineau Park road about 6 miles north of Eardley, *W. M. Bowden & W. G. Dore 132-55*; $2n =$ ca. 42 det. on one clone.

(14) *Nardus stricta* L.

Two accessions had the somatic chromosome number $2n = 30$.

Voucher specimens: Newfoundland: Cyt. No. 871 and 872, north side of St. John's, *I. J. Bassett s.n., July, 1949*; $2n = 30$ det. on two clones.

Löve and Löve (1956) examined Icelandic specimens of *N. stricta* and listed (p. 133) several counts, $2n = 26, 27, 28, 29$, and 30.

(15*a*) *Oryzopsis asperifolia* Michx.

One collection had the somatic number $2n = 48$.

Voucher specimen: Ontario: Cyt. No. 1581, Renfrew Co., 2½ miles northwest of Chalk River, *W. M. Bowden, H. A. Senn, & J. M. Gillett 17-53*; $2n = 48$ det. on one clone.

Johnson (1945) reported $2n = 46$ for this species.

(15*b*) *Oryzopsis pungens* (Torr.) Hitchc.

One collection had the somatic number $2n = 24$.

Voucher specimen : Quebec: Cyt. No. 994, Nemiscau, ¼ mile south of H. B. Co. post, *G. Spafford 68*; $2n = 24$ det. on one plant.

Johnson (1945) reported $2n = 22$ for *O. pungens*.

(15*c*) *Oryzopsis racemosa* (J. E. Smith) Ricker

One collection had the somatic number $2n = 48$.

Voucher specimen: Ontario: Cyt. No. 1760, Carleton Co., 2 miles SW. of Manotick Station, *W. M. Bowden & W. G. Dore 83-53*; $2n = 48$ det. on one clone.

Johnson (1945) reported $2n = 46$ for *O. racemosa*.

(16) *Panicum xanthophysum* A. Gray

One collection of this species was tetraploid, $2n = 36$.

Voucher specimen: Quebec: Cyt. No. 1820, west side of Gatineau Park, about 10 miles north of Eardley, *W. M. Bowden 86-54*; $2n = 36$ det. on one clone.

(17) *Phalaris arundinacea* L.

One accession was tetraploid, $2n = 28$.

Voucher specimen: Ontario: Cyt. No. 1899, Great Cloche Island, beach of North Channel, Lake Huron, *W. C. Dore et al. 16457*; $2n = 28$ det. on one plant.

Hanson and Hill (1953) determined the chromosome numbers of 186 plants of *Phalaris arundinacea*; 169 plants were tetraploid, $2n = 28$; 1 plant had $2n = 27$; 10 plants had $2n = 29$; 2 plants had $2n = 30$; 1 plant had $2n = 31$; and 3 plants had $2n = 35$.

(18*a*) *Phleum alpinum* L.

Three collections were tetraploid, $2n = 28$. Some authors have called this species *P. commutatum* Gaudin. The Linnaean type of *P. alpinum*, however, is from Lapland and this nomenclatural problem has been discussed by Bowden (1960*b*).

Voucher specimens: Newfoundland: Cyt. No. 1404, St. Anthony, *D. B. O. Savile 3005*; $2n = 28$ det. on one plant. Quebec: Cyt. No. 954 and 1026, Great Whale River, *D. B. O. Savile 694*; $2n = 28$ det. on two plants. Alaska: Cyt. No. 1348, Seward, *J. A. Calder s.n., August, 1951*; $2n = 28$ det. on one plant.

Tischler (1950) and Löve and Löve (1956) compiled many literature reports (under *P. commutatum* Gaudin) and they listed $2n = 28$ for most plants of *P. alpinum* L.

(18*b*) *Phleum pratense* L.

Ten collections were hexaploid, $2n = 42$.

Voucher specimens: Newfoundland: Cyt. No. 792, near Stephenville, *I. J. Bassett s.n., July, 1949*; $2n = 42$ det. on one clone. Cyt. No. 827; 842; and 847, Gander, *I. J. Bassett s.n., July, 1949*; $2n = 42$ det. on three clones. Labrador:

Cyt. No. 1065 and 1066, Goose Bay, *J. M. Gillett s.n., July, 1950*; $2n = 42$ det. on two clones. Mackenzie District: Cyt. No. 1248, Lower Hay River, *W. H. Lewis 2*; $2n = 42$ det. on one clone. Cyt. No. 881, Yellowknife, *W. J. Cody & J. B. McCanse 3040*; $2n = 42$ det. on one clone. Cyt. No. 923, Yellowknife, *W. J. Cody 3516*; $2n = 42$ det. on one plant. Cyt. No. 924, Yellowknife, north end of Kam Lake, *W. J. Cody & J. B. McCanse 3527*; $2n = 42$ det. on one plant.

Tischler (1950) and Löve and Löve (1956) listed many literature reports for this species and most of the counts were $2n = 42$.

(19) *Sieglingia decumbens* (L.) Bernh.

Two collections were tetraploid, $2n = 36$.

Voucher specimens: Newfoundland: Cyt. No. 876 and 879, north side of St. John's, *I. J. Bassett s.n., July, 1949*; $2n = 36$ det. on two clones.

The chromosome count $2n = 36$ for the material from Newfoundland agrees with the same chromosome number determined for some of the material of this species reported by Löve and Löve (1956, p. 128).

(20) *Sorghastrum nutans* (L.) Nash

One collection had the chromosome number $2n = 40$.

Voucher specimen: Ontario: Cyt. No. 1686, Norfolk Co., near Turkey Point, *W. M. Bowden 56-53*; $2n = 40$ det. on one clone.

Brown (1950) reported $2n = 40$ and Gould (1956) listed $n = 20$ for this species.

(21) *Spartina gracilis* Trin.

One accession was hexaploid, $2n = 42$.

Voucher specimen: Mackenzie District: Cyt. No. 1077, Salt Plain west of Fort Smith, *W. J. Cody & C. C. Loan 4578*; $2n = 42$ det. on one clone.

Church (1940) reported $2n = 42$ for a collection from Nevada.

(22) *Sphenopholis intermedia* (Rydb.) Rydb.

Three collections proved to be diploid, $2n = 14$.

Voucher specimens: Ontario, Cyt. No. 1850, Grenville Co., 2 miles east of Kemptville, *W. M. Bowden & W. G. Dore 116-55*; $2n = 14$ det. on one clone. Cyt. No. 1854, Carleton Co., between Harwood Plains and Shirley Bay, *W. M. Bowden 123-55*; $2n = 14$ det. on one clone. Cyt. No. 1563, Frontenac Co., Sharbot Lake, in moist meadow, *W. G. Dore & K. Mills 13779*; $2n = 14$ det. on two plants.

Neilsen and Humphrey (1937) reported $2n = 14$ for this species.

(23) *Sporobolus cryptandrus* (Torr.) A. Gray

One collection was tetraploid, $2n = 36$.

Voucher specimen: Alberta: Cyt. No. 1469, 5 miles SE. of Medicine Hat, *W. G. Dore & A. J. Breitung 11873*; $2n = 36$ det. on one plant.

Brown (1950) reported $2n = 36$ and Gould (1958) listed $n = 19$ for this species.

(24) *Stipa comata* Trin. & Rupr.

One collection was tetraploid, $2n = 44$.

Voucher specimen: Yukon: Cyt. No. 980, Tantalus Butte, Carmacks, *J. M. Gillett & D. A. Mitchell 3998*; $2n = 44$ det. on one plant.

Stebbins and Love (1941) reported $2n = 44$–46 for this species.

(25*a*) *Trisetum flavescens* (L.) Beauv.

One collection was tetraploid, $2n = 28$.

Voucher specimen: Alberta: Cyt. No. 1114, Waterton Lakes National Park, disturbed roadside, *W. G. Dore 12363 C*; $2n = 28$ det. on one plant.

Tischler (1950) listed literature reports of $2n = 24$ and $2n = 28$ for this species.

(25*b*) *Trisetum molle* (Michx.) Kunth

Six accessions were examined cytologically and proved to be hexaploid, $2n = 42$.

The *Trisetum spicatum* complex has recently been studied by Hultén (1959) and he listed numerous subspecies and varieties under *T. spicatum*. Böcher (1959) and Morrison (1959) found both tetraploids and hexaploids in the *T. spicatum* complex. In the present paper, Böcher's treatment is followed; he included the tetraploids under *T. spicatum* (L.) Richt. and the hexaploids under *T. molle* (Michx.) Kunth.

Voucher specimens of *T. molle*: Labrador: Cyt. No. 1020, Goose Bay, *W. B. Schofield 681*; $2n = 42$ det. on one plant. Cyt. No. 1118, North West River, *J. M. Gillett & W. I. Findlay 5536*; $2n = 42$ det. on one plant. Quebec: Cyt. No. 654, Fort Chimo, *J. A. Calder s.n., August, 1948*; $2n = 42$ det. on one clone. Cyt. No. 956, Great Whale River, near Hudson Bay Post, *D. B. O. Savile 727*; $2n = 42$ det. on one plant. Cyt. No. 1027, Great Whale River, near Hudson Bay Post, *D. B. O. Savile 648*; $2n = 42$ det. on one plant. Mackenzie District: Cyt. No. 890, Yellowknife, *W. J. Cody & J. B. McCanse 3032*; $2n = 42$ det. on one clone.

The hexaploid number $2n = 42$ has been reported for *T. molle* by Böcher (1959) and Morrison (1959).

(25*c*) *Trisetum spicatum* (L.) Richt.

Five collections were examined cytologically and the plants were tetraploid, $2n = 28$.

Voucher specimens: Franklin District: Cyt. No. 602, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n = 28$ det. on one clone. Keewatin District: Cyt. No. 703, Southampton Island, Coral Harbour, *W. J. Cody 1935*; $2n = 28$ det. on one plant. Alaska: Cyt. No. 1262, Falls Creek, Mile 229, Richardson Highway, *W. J. Cody 6308*; $2n = 28$ det. on one plant. Cyt. No. 1355, Falls Creek Mine near Kenai Lake, Kenai Peninsula, *J. A. Calder 6178*; $2n = 28$ det. on one plant. Cyt. No. 1356, Victor Creek, Kenai Lake, Kenai Peninsula, *J. A. Calder 5816*; $2n = 28$ det. on one plant.

The tetraploid number $2n = 28$ has been reported for *T. spicatum* by Böcher (1959), Morrison (1959), Tateoka (1954), Holmen (1952), Löve and Löve (1956), and Flovik (1940).

Acknowledgments

The author deeply appreciates the material that has been sent in by the collectors. He is indebted to them for preliminary identifications of some of the specimens and to Dr. W. G. Dore for detailed examinations and identifications of most of the collections.

References

BJÖRKMAN, S. O. 1951. Chromosome studies in *Agrostis*. Hereditas, **37**, 465–468.
——— 1952. Personal communication (a letter to Dr. W. G. Dore).
——— 1954. Chromosome studies in *Agrostis*. II. Hereditas, **40**, 254–258.
BÖCHER, T. W. 1959. Tetraploid and hexaploid *Trisetum spicatum* coll. A cytotaxonomical study. Botan. Tidsskr. **55**, 23–29.
BÖCHER, T. W. and LARSEN, K. 1950. Chromosome numbers of some arctic or boreal flowering plants. Medd. Grønland, **147** (6), 1–32.
BOSEMARK, N. O. 1957. Further studies on accessory chromosomes in grasses. Hereditas, **43**, 236–297.
BOWDEN, W. M. 1959. Chromosome numbers and taxonomic notes on northern grasses. I. Tribe Triticeae. Can. J. Botany, **37**, 1143–1151.
——— 1960*a*. Chromosome numbers and taxonomic notes on northern grasses. II. Tribe Festuceae. Can. J. Botany, **38**, 117–131.
——— 1960*b*. An experimental taxonomist examines the classification of grasses. Rev. can. biol. In press.
BROWN, W. V. 1948. A cytological study in the Gramineae. Am. J. Botany, **35**, 382–395.
——— 1950. A cytological study of some Texas Gramineae. Bull. Torrey Botan. Club, **77**, 63–76.
CHURCH, G. L. 1929. Meiotic phenomena in certain Gramineae I. Festuceae, Aveneae, Agrostideae, Chlorideae, and Phalarideae. Botan. Gaz. **87**, 608–629.
——— 1936. Cytological studies in the Gramineae. Am. J. Botany, **23**, 12–15.
——— 1940. Cytotaxonomic studies in the Gramineae: *Spartina*, *Andropogon* and *Panicum*. Am. J. Botany, **27**, 263–271.
DORE, W. G. Unpublished.
EHRENBERG, L. 1945. Kromosomtalen hos några kärlväxter. Botan. Notiser, 1945, 430–437.
FLOVIK, K. 1940. Chromosome numbers and polyploidy within the flora of Spitzbergen. Hereditas, **26**, 430–440.
GOULD, F. W. 1956. Chromosome counts and cytotaxonomic notes on grasses of the tribe Andropogoneae. Am. J. Botany, **43**, 395–404.
——— 1958. Chromosome numbers in southwestern grasses. Am. J. Botany, **45**, 757–767.
HANSON, A. A. and HILL, H. D. 1953. The occurrence of aneuploidy in *Phalaris* spp. Bull. Torrey Botan. Club, **80**, 16–20.
HEDBERG, O. 1958. Cyto-taxonomic studies in Scottish mountain plants, notably *Deschampsia caespitosa* (L.) PB., s. lat. Svensk. Botan. Tidskr. **52**, 37–46.
HITCHCOCK, A. S. 1951. Manual of the grasses of the United States. 2nd ed. *Revised by* A. Chase. U.S. Dept. Agr. Misc. Publ. No. 200.
HOLMEN, K. 1952. Cytological studies in the flora of Peary Land, North Greenland. Medd. Grønland, **128** (5), 1–40.
HULTÉN, E. 1942. Flora of Alaska and Yukon. Vol. 2. Gleerup, Lund.
——— 1959. The *Trisetum spicatum* complex. Svensk. Botan. Tidskr. **53**, 203–228.
JOHNSON, B. L. 1945. Cyto-taxonomic studies in *Oryzopsis*. Botan. Gaz. **107**, 1–32.
JOHNSSON, H. 1941. Cytological studies in the genus *Alopecurus*. Lunds Univ. Arsskr. N. F. Avd. 2, **37** (3) or Kgl. Fysiograf. Sällskap. Lund, Handl. N. F. **52** (3), 1–43.
JONES, K. 1956. Species differentiation in Agrostis I. Cytological relationships in *Agrostis canina* L. J. Genetics, **54**, 370–376.
JØRGENSEN, C. A., SØRENSEN, TH., and WESTERGAARD, M. 1958. The flowering plants of Greenland. A taxonomical and cytological survey. Biol. Skr. Dan. Vid. Selsk. **9** (4), 1–172.
LAPSHIN, M. M. 1939. [Concerning the systematics of the grasses of the Gorkovskoy Oblast.] Proc. Gork. State Ped. Inst. **4**, 135–159.
LINNAEUS, C. 1753. Species Plantarum. 1st ed. Holmiae.
LÖVE, A. and LÖVE, D. 1956. Cytotaxonomical conspectus of the Icelandic flora. Acta Horti Gotoburgensis, **20** (4), 65–290.
MORRISON, M. E. S. 1959. Interrelationships of some of the species of *Trisetum* from North America and Europe. Can. J. Genetics Cytology, **1**, 84–88.
MOSS, E. H. 1959. Flora of Alberta. University of Toronto Press.

MYERS, W. M. 1947. Cytology and genetics of forage grasses. Botan. Rev. **13**, 319–421.
NEILSEN, E. L. and HUMPHREY, L. M. 1937. Grass studies I. Chromosome numbers in certain members of the tribes Festuceae, Hordeae, Aveneae, Agrostideae, Chlorideae, Phalarideae and Tripsaceae. Am. J. Botany, **24**, 276–279.
NYGREN, A. 1946. The genesis of some Scandinavian species of *Calamagrostis*. Hereditas, **32**, 131–262.
——— 1954. Investigations on North American *Calamagrostis*. I. Hereditas, **40**, 377–397.
SOKOLOVSKAYA, A. P. 1938. A caryo-geographical study of the genus *Agrostis*. Cytologia, **8**, 452–467.
SOKOLOVSKAYA, A. P. and STRELKOVA, O. S. 1938. Polyploidy in the high mountain regions of Pamir and Altai. Comp. rend. acad. sci. U.S.S.R. **21**, 68–71.
STEBBINS, G. L., JR., and LOVE, R. M. 1941. A cytological study of California forage grasses. Am. J. Botany, **28**, 371–382.
STUCKEY, I. H. and BANFIELD, W. G. 1946. The morphological variations and the occurrence of aneuploids in some species of *Agrostis* in Rhode Island. Am. J. Botany, **33**, 185–190.
TATEOKA, T. 1954. Karyotaxonomy in Poaceae II. Somatic chromosomes of some species. Cytologia, **19**, 317–328.
——— 1955. Karyotaxonomy in Poaceae III. Further studies of somatic chromosomes. Cytologia, **20**, 296–306.
TISCHLER, G. 1950. Die Chromosomenzahlen der Gefässpflanzen Mitteleuropas. W. Junk. 'S-Gravenhage.
WET, J. M. J. DE. 1954. The genus *Danthonia* in grass phylogeny. Am. J. Botany, **41**, 204–211.
——— 1960. Chromosome numbers and some morphological attributes of various South African grasses. Am. J. Botany, **47**, 44–49.

THE GENUS HETEROCHAETELLA[1]

E. ROBENA LUCK-ALLEN

Abstract

Heterochaetella is retained as a separate genus to include three species. *Heterochaete dubia* Bourd. & Galz. is the type. *Heterochaetella bispora*, found on decayed wood in Ontario, is proposed as a new species. *Heterochaetella brachyspora*, treated as a variety of *H. dubia* by Bourdot and Galzin, is raised to specific rank. A redescription of the genus is given as well as descriptions and illustrations of the three species. *Heterochaetella crystallina* Bourd. is considered a synonym of *Stypella papillata* Möller and not congeneric with *H. dubia*.

Introduction

Heterochaetella was first described as a subgenus by Bourdot (2) in 1920 in order to accommodate species of *Sebacina* with cystidia, the gloeocystidiate species having been previously segregated into the genus *Bourdotia* which was established in 1913 by Bresadola and Torrend.

In his publication of 1920, Bourdot (2) established *Heterochaetella* as a subgenus for the previously described species, *Heterochaete dubia*. This is the only previously described species of *Heterochaete* to be included in the subgenus *Heterochaetella*. In this same publication (2) Bourdot added a second newly described species, *H. crystallina*. In 1928, Bourdot and Galzin (4) raised the subgenus to generic status. There is general agreement (6, 7, 8, 15, 18) that *Heterochaete dubia* should be regarded as the type of the genus *Heterochaetella*.

The taxon has received various treatments by investigators interested in the group. The combination *Sebacina dubia* (Bourd. & Galz.) Bourd. first appeared in 1922. Burt (5) treated *Heterochaetella* as a subgeneric segregate of *Sebacina* as did also Rogers (19). Killermann (9) included it as a subgenus of *Heterochaete*. In 1941, McGuire (15) recognized *Heterochaetella* as a section of *Sebacina*. Martin (12) followed McGuire in his treatment of the category both in the 1944 publication and in his revision of 1952 (14). Ervin (8) recognized the generic status of *Heterochaetella* as outlined by Bourdot and Galzin (4). Pilát (18) also recognized *Heterochaetella* as a genus in his elaborate treatment of 1957.

Discussion

In 1892 (17) the genus *Heterochaete* Pat. was published for species of *Sebacina* with short, cylindrical emergencies which project from the hymenium and which consist of compacted, sterile hyphae with or without cystidia. It was in this category that the epithet *dubia* first found its position (3). According to Bodman (1) the species included here are coriaceous to gelatinous with the hymenium punctuated by irregularly distributed, infrequent or numerous sterile

[1]Manuscript received April 8, 1960.
Contribution from the Department of Botany, University of Toronto, Toronto, Ontario. Portion of a thesis presented at the University of Toronto in partial fulfilment of the requirements of the degree of Doctor of Philosophy.

pegs. *Heterochaete dubia* Bourd. & Galz. certainly bears no relationship to any of the known species of *Heterochaete* and the establishment of the subgenus *Heterochaetella* in order to accommodate it in 1920 was fully justified. The typical cystidia of *H. dubia* are certainly not seen in any of the described species of *Heterochaete*.

Bourdot and Galzin in 1928 visualized the confusion engendered by the nature of the cystidia of *H. dubia* and pointed to the cystidial analogy in species of *Peniophora glebulosa*, *P. subalutacea*, and *Odontia barba-jovis*. Other than the superficial resemblances of these cystidial elements, the species mentioned above bear no likeness to *H. dubia* since they are homobasidiomycetes with typical clavate basidia and *H. dubia* a heterobasidiomycete with cruciate-septate basidia.

As described by Bourdot and Galzin (4), *Heterochaetella* included species whose fructifications were effused, firm-gelatinous to mucous-pruinose, and with cystidia thin- or thick-walled, prolonged, and emergent. The description of the genus aptly accommodates *H. dubia* but not *H. crystallina*. *Heterochaetella dubia* is an interesting form with a hispid hymenium composed of individual or fasciculate cystidia bearing blunt apices, dilated lumina, and thickened walls. *H. crystallina* was originally described as substipitate, lobed, clavariform, or interrupted. The fructification appeared as a crystalline pellicle surrounded by a network which on drying resembled a delicate lace thread (2). An examination of an authentic collection of *H. crystallina* Bourd. from Paris was found to be non-congeneric with *H. dubia* and as such is being removed from *Heterochaetella* by this investigator. It shows no kinship to *H. dubia* whatever. In fact, as McGuire (15) suggested, it proves to be identical with *Stypella papillata* Möller (sensu Martin) (11). It is proposed that *H. crystallina* be removed from *Heterochaetella* and placed in synonomy with *Stypella papillata* Möller.

In 1957, Ervin (8) recommended the segregation of *H. dubia* from species of *Sebacina* mainly on the basis of the presence of the characteristic cystidia. Olive (16), in 1958, argued for a retention of the McGuire concept—that of maintaining *Heterochaetella* as a section of *Sebacina*. Rogers (19) earlier had expressed the opinion that there is little justification for erecting a new genus "whenever a group of species is encountered having some distinctive hymenial development". He maintains that *Heterochaetella* has affinities with both arid and soft species of *Eusebacina*, and thus treats *Heterochaetella* as a subgenus.

Like Rogers, Olive (16) feels that the possession of cystidia or gloeocystidia is to be regarded as only one of a number of characters which singly is not sufficient to warrant the elevation of the taxon to the rank of genus. In order to support his beliefs, Olive (16) points to the fact that a new species described by him from Tahiti, *Sebacina minima*, has thin-walled cystidia as does also *Sebacina psilochaete* (Bourd. & Galz.) Olive, and that these cystidia are less bristle-like than those of *H. dubia*. He feels that the type of elements common to the species mentioned above are probably intermediate between the kind of gloeocystidia which one sees in *Bourdotia* and the hispid cystidia typical

of *H. dubia*. He further maintains that *S. minima* and *S. psilochaete* are transitional members linking *Heterochaetella* with *Bourdotia* too closely for generic segregation.

The writer (10) believes that *Heterochaetella* with its large, spreading, interrupted fruit body and hispid hymenium should be separated from *Sebacina*. In this she is in accord with Ervin (8). It is evident that *H. dubia* stands out singularly from other species of *Sebacina*. The emergent, thick-walled cystidia, which do not dissolve in KOH, are too distinctive to regard as a character of minor importance. Such characteristic elements do not exist elsewhere within the family Tremellaceae. In *Exidiopsis sublilacina* (Martin) Ervin, and *Exidiopsis podlachica* (Bres.) Ervin, there are thin-walled, subulate sterile elements which project, at times, up to 30 μ beyond the basidia. Like Olive (16), the writer questions whether one could consider these elements as structures closely related to the cystidia of *H. dubia* or more closely akin to the gloeocystidia of species of *Bourdotia* or as something entirely different from both of these. The writer is of the opinion that the sterile elements seen in *E. sublilacina* and *E. podlachica* are not related to those of *H. dubia*. Might it not be possible to conceive that species of *Heterochaetella* may have had a separate line of development?

Description of Genus

HETEROCHAETELLA (Bourd.) Bourd. & Galz. Hymén. Fr. 51. 1928.

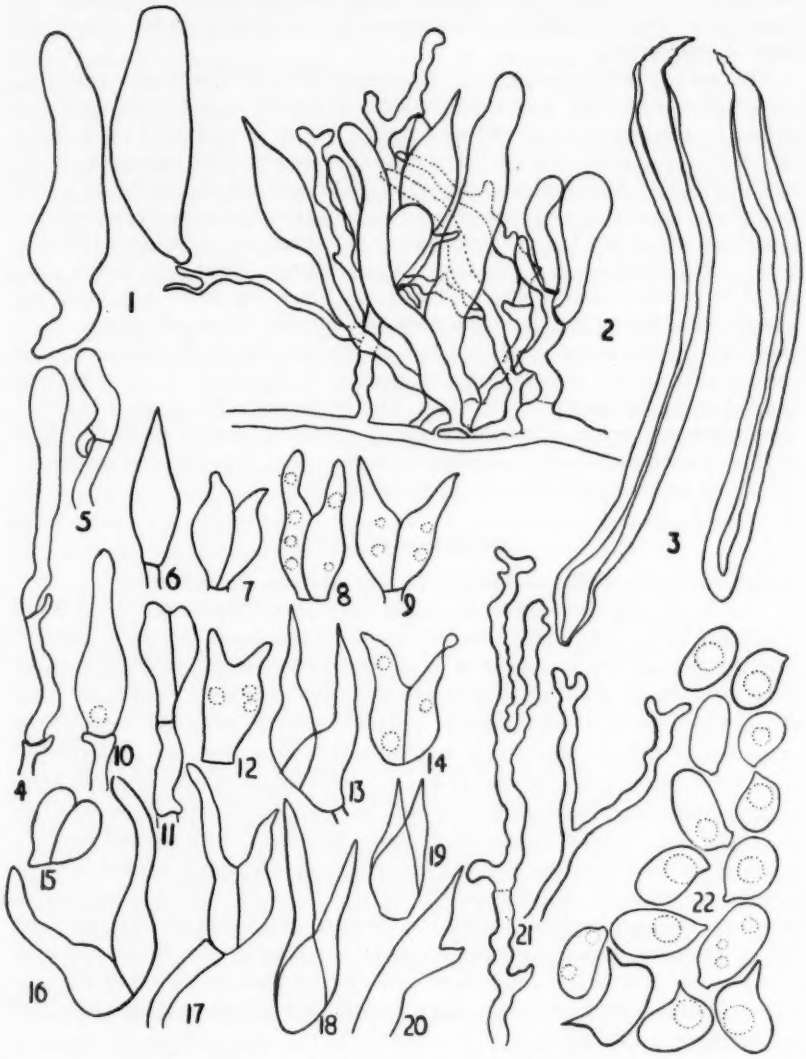
Sebacina subg. *Heterochaetella* Bourd. Trans. Brit. Mycol. Soc. 7: 53. 1920.

Effused, resupinate, indeterminate, firmly membranous, drying to a waxy-araneous, rimose, or crust-like layer, typically discontinuous, rarely continuous; hymenium punctuated with infrequent, numerous hispid, fimbriate tufts which anastomose or with minute, isolated, conical, spine-like outgrowths composed of cystidia and fertile hyphae which arise from an agglutinated basal layer; occasionally drying to a thin almost imperceptible pruinose film; hymenial elements arising directly from the substrate; dendrophyses distinct or gelatinized; cystidia thick-walled, blunt, emergent, with dilated lumina; lumen often with basal occlusion; with stellate or irregularly shaped crystals adhering to elements or dispersed throughout the subhymenium; probasidia ovoid to clavate; basidia globose to obovate longitudinally divided into two to four cells, each cell bearing a tubular protosterigma or short sterigma; spores varying in shape from short-cylindric, or oblong, flattened on one side to obovate, oval, or spherical with prominent apiculus, germinating by repetition.

Type species: *Heterochaete dubia* Bourd. & Galz.

Key to Species

1. Basidia 2-celled, dendrophyses prominent..... *H. bispora*
1. Basidia 4-celled, dendrophyses gelatinized or absent.....2
   2. Spores oblong, laterally depressed, 6–9×3–5 μ..... *H. dubia*
   2. Spores ovoid or spherical 5–6×4–5 μ..... *H. brachyspora*



FIGS. 1–22. *Heterochaetella bispora*. Fig. 1. Two gloeocystidia-like elements. Fig. 2. Reconstructed hymenium showing sterile and fertile elements. Fig. 3. Cystidia. Figs. 4, 5. Young probasidia with clamp connections. Figs. 6, 10. Basidia, each with one sterigma. Figs. 6–20. Showing character of basidia. Fig. 21. Tortuous dendrophyses. Fig. 22. Basidiospores, three germinating. Reproduced at approximately ×1600.

Description of Species

HETEROCHAETELLA **bispora** sp. nov. (Figs. 1–22)

Fructificatio sicca crustam effusam continuam ceraceam tuberculatam formans, vix visibilis, superficie alba pruinosa; maturis partibus interrupta interdum paene farinacea, plerumque rava; basidia in zona angusta paraphysibus et dendrophysibus interspersis (Fig. 2); cystidia (Fig. 3) flexibilia aut recta luminibus, angustis, singula, dispersa, 50–160×4–5 μ; elementa gloeocystidiorum similia (Fig. 1) tenui-tunicata aut gracilia aut brevia turgidaque aut sine forma 24–36×3–7.5 μ; dendrophyses tortuosae (Fig. 21) numerosae, 1.5–2 μ diam. alba crystalla interspersa; probasidia primo clavata (Figs. 4, 5), 4–4.5×9 μ, deinde obovata, 7–9×4.5–6 μ; basidia 2-cellularia (Figs. 7, 8, 9, 11, 13–19) (modo uno sterigmate, Figs. 6, 10) obovata, 7–9×4.5–6 μ interdum globosa 7.5 μ, septum obliquum (Figs. 11, 17); protosterigmata 1.5–2 μ diam. usque ad 22 μ longitudine; sporae cylindraceo-oblongae, interdum obovatae, 6–9(–10)×3–4.5 μ, per repetitionem germinantes (Fig. 22).

Fructification, when dry, an effused continuous, ceraceous tuberculate crust, inconspicuous, drab, covered by a faint, whitish bloom, interrupted in older areas, verging toward farinaceous, rarely appearing rimose; varying from cinnamon buff to clay color (R); composed of a basal layer of indistinct horizontal hyphae, an intermediate layer of toruloid sterile and fertile hyphae, and a narrow compact zone of basidia interspersed with simple paraphysoids and dendrophyses (Fig. 2); cystidia (Fig. 3) with narrow lumina, generally occurring singly, scattered, or rarely in fascicles of four to five, flexible or straight, measuring 50–160×4–5 μ and projecting 50–60 μ beyond basidia; gloeocystidia-like elements (Fig. 1) thin-walled, slender, long or short, ventricose with blunt apices or otherwise irregular, emanating below the subhymenium, non-emergent, in rare cases projecting only slightly, staining red in KOH–Phloxine, measuring 24–36×3–7.5 μ; dendrophyses (Fig. 21) numerous, tortuous, 1.5–2 μ often arising from clamps and coming off branches of the fertile stalks, occasionally with a few simple paraphysoids intermixed, non-emergent; fertile hyphae bearing probasidia distinct, measuring 1.5–3 μ in diameter, staining red in KOH–Phloxine while that subtending mature basidia indistinct and not staining red in KOH–Phloxine; with numerous spinulose, stellate, or otherwise irregularly shaped crystals scattered throughout the subhymenium frequently adhering to elements or free in the field; probasidia at first clavate, 4–4.5×9 μ with basal clamp some distance back along the fertile hyphae (Figs. 4, 5), finally becoming obovate; basidia two-celled, guttulate (Figs. 7, 8, 9, 11, 13–19) often with only one sterigma (Figs. 6, 10), measuring 7–9×4.5–6 μ, with septum oblique (Figs. 11, 17) and separating out from apex downward (Figs. 15, 16); protosterigmata 1.5–2 μ in diameter up to 22 μ in length with sterigmata blunt or verging toward subulation; spores oblong, cylindrical, flattened on one side, widest toward the upper extremity and narrowing at the apiculate end, occasionally obovate, guttulate, 6–9(–10)×3–4.5 μ, germinating by repetition (Fig. 22).

This is a very interesting species with two-celled basidia which clearly seems related to *H. dubia* through the character of its growth habit, nature of the cystidia, and morphology of its basidia and spores. Sterile elements strongly suggestive of gloeocystidia are quite common in this species. The writer suspects that these are possibly stages in the development of the cystidia. Further study is needed in order to verify this point. Only one collection examined: TYPE: *Ontario*; Brant County: south of New Durham Aug. 30, 1937 on *Ulmus*. R. F. Cain (TRTC 17594).

HETEROCHAETELLA DUBIA (Bourd. & Galz.) Bourd. & Galz. Hymén. Fr. 51. 1928. (Figs. 23–36)

Heterochaete dubia Bourd. & Galz. Bull. Soc. Myc. Fr. 25: 30. 1909.

Sebacina dubia (Bourd. & Galz.) Bourd., Ass. Fr. Av. Sc. 45: 576. 1922.

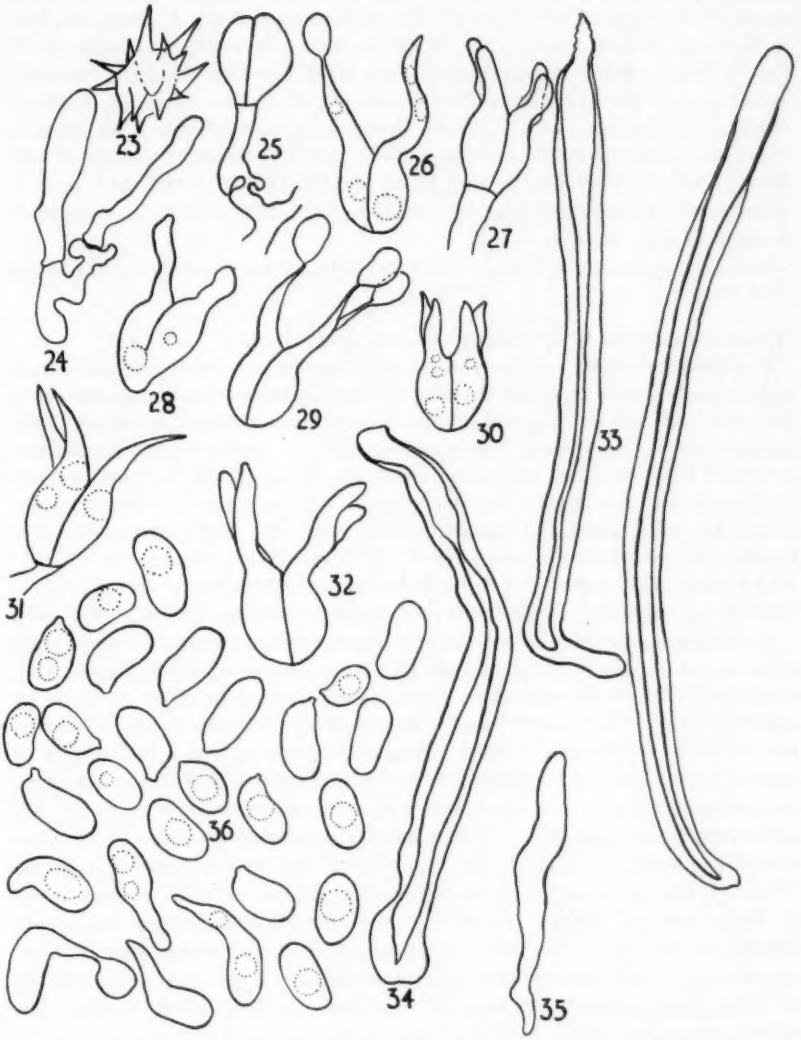
When fresh, effused, mucous-waxy, pure white, frosted, pustulate, diaphanous, confluent, separable, thinning out at margin, verging toward farinaceous, drying to a coriaceous interrupted arid crust, varying from light buff (R) to Capucine buff (R) darkening to ochraceous tawny or tawny (R) with prominent hispid projections occurring singly or in tufts scattered over the hymenial surface or occasionally characterized by a thin, inconspicuous, minutely hispid, whitish, granular layer; hymenium composed of pillars of thick-walled cystidia, straight or flexible occurring singly or in fascicles with sterile and fertile hyphae forming a compact unit around projecting cystidia and with numerous spinulose, stellate, or irregularly shaped crystals free or adhering to elements (Fig. 23), cystidia measuring 3–6×50–200 μ (Figs. 33, 34, 35) with blunt or attenuated, dilated, hispid apices, projecting up to 100 μ beyond basidia and non-dissolvent in KOH; fertile hyphae 1.5–2 μ, clamp-bearing; probasidia elongate to clavate (Fig. 24), 5–5.6×7.5–9 μ; basidia globose to subglobose, finally obovate, 6–9×7.5–10.5 μ; when globose mostly 6–7.5 μ, at first two-celled finally four-celled, with apices of sterigmata blunt or subulate, measuring 1.5–2 μ in diameter, up to 20 μ long (Figs. 25–32); spores oblong-cylindrical, laterally depressed, attenuated at apiculate end, 3–5×(5–)6–9 μ, germinating by repetition (Fig. 36).

Heterochaetella dubia is being treated as a distinct unit on the basis of the spore character. Bourdot and Galzin (4) recognized several varieties of the species, delineating primarily on the basis of spore shape and character of the cystidia. Using as a yardstick the description and illustration provided for *H. dubia* by these authors (4) and after examining numerous collections of the species, the writer has arrived at her concept of this species. The spores are regarded as distinctive, either oblong or cylindrical in shape.

Type: France

Specimens Examined

ONTARIO: Algonquin Park: Brewer Lake: Sept. 5, 1939, on *Fagus grandifolia*, R. F. Cain (TRTC 15999); W. of Killarny camp: Sept. 17, 1938, *Betula*, R. F. Cain (TRTC 23477); Oxbow Lake: Aug. 13, 1941, on *Acer*, R. F. Cain (TRTC 18226); Brant County: SE. of Hatchley: Sept. 25, 1937, deciduous



FIGS. 23–36. *Heterochaetella dubia*. Fig. 23. "Stellate" crystal. Fig. 24. Young probasidium with lateral proliferation. Figs. 25–32. Basidia in various stages of differentiation and showing character of sterigmata. Figs. 33, 34. Thick-walled cystidia. Fig. 35. Cystidium in early stage of development. Fig. 36. Basidiospores, five germinating. Reproduced at approximately ×1600.

wood, R. F. Cain (TRTC 11740); New Durham: Sept. 2, 1938, decaying wood, R. F. Cain (TRTC 13941); SE. of Hatchley: Sept. 5, 1941, on *Acer*, R. F. Cain (TRTC 18228); SE. Hatchley: Oct. 10, 1938, on *Ulmus*, R. F. Cain (TRTC 13550); York County: East of Maple: Oct. 24, 1936, decaying wood, R. F. Cain (TRTC 31688); Woods W. of Maple: Sept. 22, 1946, on *Fagus*, H. S. Jackson (TRTC 21256); Frontenac County: Silver Lake: Sept. 1, 1941, deciduous wood, R. F. Cain (TRTC 18195); Renfrew County: Chalk River: Sept. 13, 1939, on *Populus*, H. S. Jackson (TRTC 15987).

QUEBEC: St. Dorothée: near Montreal: Aug. 26, 1941, deciduous tree, H. S. Jackson (TRTC 33765).

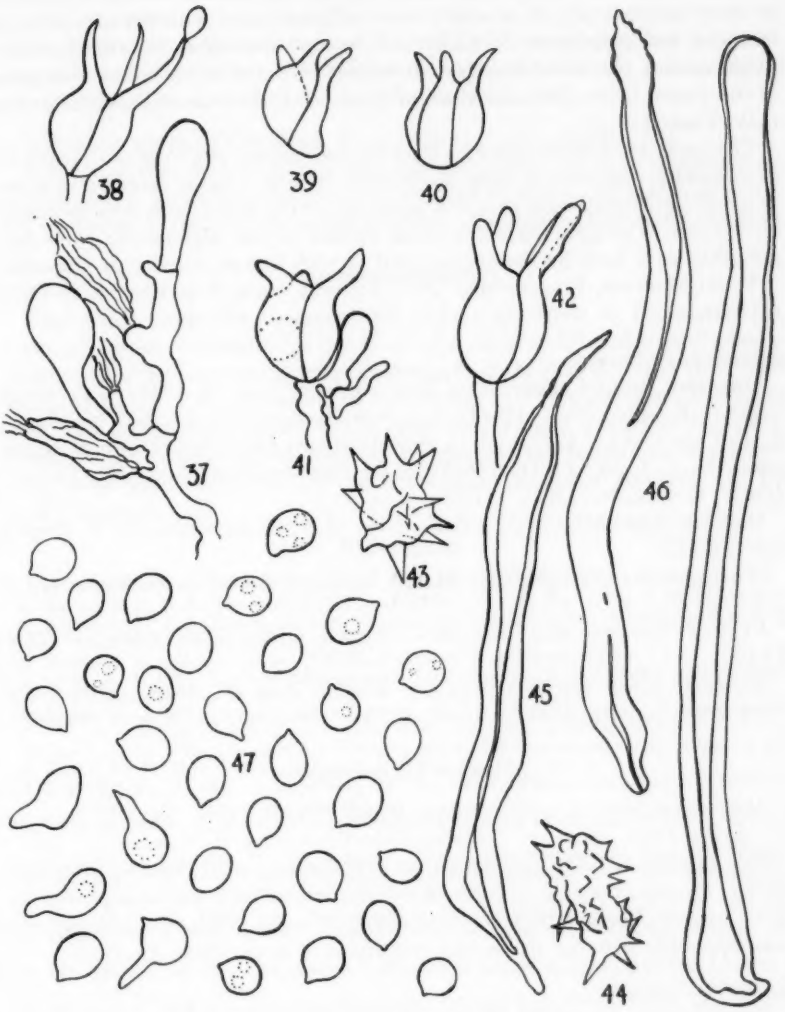
SWEDEN: Upland near Upsala: Sept. 29, 1934, rotten birch trunk, S. Lundell 1167 (BPI).

HETEROCHAETELLA **brachyspora** sp. nov. (Figs. 37–47)

Fructificatio modo crustam hyalinam continuam ceraceam cum projectionibus spinosis formans aut singulis aut fimbriatis fasciculis emergentibus, nunc stratum albulum vel fulvum in partibus antiquioribus interruptum; stratum inferius ex obscuris hyphis nodoso-septatis, 1.5–2 μ diam. constitutum, in medio stratum alium obscurum crystallis (Figs. 43, 44) et zona angusta hypharum ad 2 μ diam., cystidia singula vel fasciculata, crasse tunicata (Figs. 45, 46); probasidia primo clavata (Fig. 37), vel globosa, 6–7.5 μ; basidia 2–3 cellularia, deinde 4-cellularia (Figs. 38–42), obovata 6–9×7.5–10.5 μ; sterigmata subulata 1.5–3 μ diam. usque ad 15 μ longa; sporae ovoidae 5–6×4–5 μ interdum subglobosae per repetitionem germinantes (Fig. 47).

Fructification varying from a hyaline, imperceptible, continuous waxy crust to a cream or pale ochraceous buff (R) layer darkening to clay color (R), embedded with numerous white crystals; interrupted in older portions by spine-like projections which emerge singly or in fimbriate tufts, thinner at the margins and verging toward farinaceous; composed of a basal layer of agglutinated indistinct horizontal clamp-bearing hyphae, 1.5–2 μ in diameter, an indistinct intermediate layer with scattered stellate crystals (Figs. 43, 44) and a narrow compact zone of fertile hyphae measuring up to 2 μ in diameter which is clearly distinguishable immediately below the basidia; cystidia (Figs. 45, 46) occurring singly or in fascicles, flexible or stiff, projecting up to 100 μ beyond basidia, measuring 60–150×3–6 μ with walls thickened, apices dilated, blunt, at times tapering off into fine thread-like bristles; probasidia at first clavate (Fig. 37) then globose, when globose measuring 6–7.5 μ, basidia mostly obovate, at first two- to three-celled, finally four-celled, measuring 6–9×7.5–10.5 μ (Figs. 38–42); sterigmata 1.5–3 μ up to 15 μ long, subulate or blunt; spores ovoid sometimes almost globose, guttulate, apiculate, attenuated at base, measuring 5–6×4–5 μ, germinating by repetition (Fig. 47).

This species resembles *H. dubia* in most respects, but is being separated from it primarily on the basis of the spore shape. In *H. brachyspora* the spores are distinctly ovoid while in *H. dubia* they are oblong and laterally depressed.



FIGS. 37–47. *Heterochaetella brachyspora*. Fig. 37. Young probasidia and old, collapsed basidia. Figs. 38–42. Three- and four-celled basidia. Figs. 43, 44. Stellate crystals. Figs. 45, 46. Thick-walled cystidia. Fig. 47. Basidiospores, five germinating. Reproduced at approximately ×1600.

Heterochaetella dubia is rather complex, made up (sensu Bourdot & Galzin) of three varieties (4), all of which have included forms with the exception of *H. dubia* var. *psilochaeta*. McGuire (15) incorporated these varieties into the single species, *H. dubia*. Martin (14) follows McGuire in his latest treatment of the taxon. Olive (16) raised one of these, *H. dubia* var. *psilochaeta*, to the rank of species.

The name *brachyspora*, used by Bourdot and Galzin, as a form of the variety *mesochaeta* is discussed in their publication (4) and is being retained in order to avoid the introduction of a new name which the writer feels is unnecessary in this case. The combination is being treated as new because the name was published as a form by the authors and as such has no validity as a species.

Type: Marvivo, France. May 1925, decayed wood, A'de Crozals 70 (BPI 171289).

Specimens Examined

ONTARIO: Brant County: S. of New Durham: July 12, 1937, on decaying wood, R. F. Cain (TRTC 12824); S. of New Durham: July 13, 1937, on *Ulmus*, R. F. Cain (TRTC 12822); S. of New Durham: July 15, 1937, on decaying wood, R. F. Cain (TRTC 12825); S. of New Durham: Aug. 30, 1937, on *Ulmus*, R. F. Cain (TRTC 11739).

OREGON: Alsea Mts: Mary's Peak: Nov. 27, 1936, on conifer, D. P. Rogers 217 (TRTC).

WASHINGTON: Olympic Mts: May 9, 1939, on *Alnus*? A. H. Smith 11318 (TRTC).

FRANCE: Marvivo: May 1925, decayed wood, A'de Crozals 70 (BPI 171289) TYPE.

AUSTRIA: Tirol: Karwendel gebeit: Rontal: Aug. 24, 1928, on decaying wood *Picea excelsa*, V. Litschauer 37, Ex. Herb. D. P. Rogers (TRTC).

Species Excludendae

1. HETEROCHAETELLA CRYSTALLINA Bourd. Trans. Brit. Mycol. Soc. 7: 50–54. 1921.

This species is being considered as synonymous with *Stypella papillata* Möller. Examinations of an authentic collection of *H. crystallina* proved to be in no way different from *Stypella papillata*, sensu Martin, and compares most favorably with our herbarium collections of *S. papillata*.

Specimens Examined

Heterochaetella crystallina Bourd. FRANCE: l'Aveyron: Causse Noir: Nov. 22, 1914, M. Galzin 16765 (Ex. Herb. H. Bourdot P.C.).

Stypella papillata Möll. ONTARIO: Lake Temagami: Bear Island: July 23, 1936, on *Pinus strobus*, R. Biggs (TRTC 13487); Bear Island: Aug. 1, 1936, on *Abies balsamea*, R. Biggs (TRTC 13493); Koko Bay: Aug. 18, 1945, on *Thuja*, H. S. Jackson (TRTC 22102); Paradis Bay: Aug. 7, 1936, on *Pinus*, R. Biggs (TRTC 13485). MASSACHUSETTS: Springfield: Aug. 16, 1943, on log of *Pinus strobus*, A. M. and D. P. Rogers 1017 (TRTC).

2. SEBACINA PSILOCHAETA (Bourd. & Galz.) Olive, Bull. Torrey Botan. Club, 85: 89. 1958.

A variety of *H. dubia* raised to specific rank by Olive and described as possessing thin-walled cystidia. No collections were examined but it is doubtful whether this species should be included in *Heterochaetella*.

3. SEBACINA MINIMA Olive, Bull. Torrey Botan. Club, 85: 89. 1958.

A tropical species with thin-walled cystidia similar to those described for *S. psilochaeta*. Tropical forms were not included in this study but, in any case, the character of the cystidia would preclude this species from *Heterochaetella*.

Acknowledgments

The writer would like to express her sincere gratitude to Professor R. F. Cain, who made it possible for her to do this project, directed it, and gave so generously of his time and knowledge throughout the period. She is very much indebted to Dr. J. A. Stevenson of the Bureau of Plant Industry, Beltsville, Md., and to Dr. Roger Heim of the Muséum National d'Histoire Naturelle, Paris, for the loan of critical material. In addition, she would like to acknowledge with great appreciation the invaluable criticisms rendered by Dr. L. K. Weresub.

References

1. BODMAN, SR. MARY C. 1952. The genus *Heterochaete*. Lloydia, **15**, 193–205.
2. BOURDOT, H. 1920. Two new basidiomycetes. Brit. Mycol. Soc. Trans. **7**, 50–54.
3. BOURDOT, H. and GALZIN, A. 1909. Hyménomycètes de France. (Héterobasidiés). Bull. Soc. Myc. Fr. **25**, 30–31.
4. BOURDOT, H. and GALZIN, A. 1928. Hyménomycètes de France. Paris.
5. BURT, E. A. 1926. The Thelephoraceae of North America. XV. *Sebacina*. Ann. Missouri Botan. Garden, **13**, 334–339.
6. DONK, M. A. 1931. Revisie van de Nederlandse Heterobasidiomycetae en Homobasidiomycetae-Aphyllophoraceae. I. Med. Nederl. Mycol. ver. **18–20**, 95–108.
7. DONK, M. A. 1958. The generic names proposed for Hymenomycetes VIII. Taxon. **7**, 202.
8. ERVIN, M. D. 1957. The genus *Sebacina*. Mycologia, **49**, 118–123.
9. KILLERMANN, S. 1928. Tremellineae. *In* Die Naturl. Pflanzenfam. 2nd ed. *Edited by* Engler and Prantl. Vol. 6. p. 113.
10. LUCK-ALLEN, E. R. 1956. A study of species of *Sebacina* in Ontario. M.A. Thesis, Dept. of Botany, Univ. of Toronto, Toronto, Ontario.
11. MARTIN, G. W. 1934. The genus *Stypella*. Univ. Iowa Studies Nat. Hist. **16**, 143–151.
12. MARTIN, G. W. 1944. The Tremellales of the north central United States and adjacent Canada. Univ. Iowa Studies Nat. Hist. **18**, 34–37.
13. MARTIN, G. W. 1945. The classification of the Tremellales. Mycologia, **37**, 527–540.
14. MARTIN, G. W. 1952. Revision of the north central Tremellales. Univ. Iowa Studies Nat. Hist. **19**, 44–47.
15. MCGUIRE, J. M. 1941. The species of *Sebacina* of temperate North America. Lloydia, **4**, 1–32.
16. OLIVE, L. S. 1958. The lower Basidiomycetes of Tahiti. Bull. Torrey Botan. Club, **85**, 89–109.
17. PATOUILLARD, N. and LAGERHEIM, G. DE. 1892. Champignons de l'Equateur. Bull. Soc. Myc. Fr. **8**, 120.
18. PILÁT, A. 1957. Ubersicht der europäischen Auriculariales und Tremellales unter besonderer berücksichtigung der tschechoslowakischen arten. Sebacinoideae. **13B** (4), 173–174.
19. ROGERS, D. P. 1933. Some noteworthy fungi from Iowa. Univ. Iowa Studies Nat. Hist. **15**, 9–12.

A PALYNOLOGICAL STUDY RELATING TO THE PLEISTOCENE TORONTO FORMATION[1]

N. W. RADFORTH AND J. TERASMAE

Abstract

The present palynological study is used as a new approach to determine the stratigraphical and chronological position of the Pleistocene interglacial deposits, the Toronto formation, exposed in sections at Toronto, Ontario, Canada.

An interpretation of lithology, combined with evidence supplied by the pollen and spore assemblages, indicates that the Don beds, the lower member of the Toronto formation, belong to the Sangamon Interglacial. The Scarborough beds, the upper member of the formation, were deposited during a nonglacial interval, cooler than the present. Conformity between the two members is questioned on grounds of lithological and palynological evidence.

Results of palynological studies, as indicated by this investigation, can be used for interpretation of the mode of deposition of certain laminated sediments and for reconstruction of past types of landscape.

Introduction

In the paleobotanical realm the authors have recognized a twofold problem bearing on age relations and the stratigraphical sequence of the Pleistocene interglacial deposits at Toronto, best exposed in the Don Valley Brickyard quarry and in the Scarborough Bluffs along the shore of Lake Ontario. One was provided by the question of whether there existed in these beds any palynological evidence sufficiently organized to be of pertinent value. The other, a more fundamental one, was the extraction of this evidence, supposing it existed, in a form in which it could be used to stratigraphical and perhaps paleoecological advantage. Particularly, it was hoped it could be used to diagnose sedimentary sequence and origin either on a local basis or perhaps for purposes of wider comparison of events. Although it was not presumed that these problems could be solved in one investigation, it was thought that paleobotanical exploration to provide an organized approach might serve as an immediate objective.

There were two unrelated circumstances which in some measure controlled the initiation of the study. One was the discovery of plant fragments uncovered by the excavation for the Toronto Rapid Transit Subway which cut through Pleistocene deposits similar to those of the interglacial Toronto formation, but for which stratigraphical relationship remained partly in doubt. The other was an immediate need to provide information on the genesis of certain laminated beds encountered in sections of the Don and the Scarborough members of the formation. True varving, for example, was sometimes difficult to differentiate from other sedimentary phenomena, visually resembling varves.

It should be explained that although organic remains from the Toronto formation have long been known (Hinde 1878), only the macroscopic ones have been investigated. Even collectively, it is questionable whether these provide enough suggestion to afford complete satisfaction for matters of

[1]Manuscript received January 27, 1960.
Contribution from the Geological Survey of Canada, Ottawa, Ontario.

stratigraphical correlation. Therefore, it was determined from the outset to emphasize the role of microfossils if they were procurable from the mineral matrix constituting the beds.

Acknowledgments

The writers are indebted to Mr. Robert F. Legget, Director, Division of Building Research, National Research Council, for making it possible not only for them to work on the Toronto Rapid Transit Subway samples, but also for soliciting their interpretation of this material. They are also grateful to Dr. H. Godwin, Sub-department of Quaternary Research, Cambridge University, who has helpfully scrutinized the first draft manuscript and who made suggestions concerning the co-ordination of palynological data, etc. To Miss Jean Evel, Department of Biology, McMaster University, thanks are also due for editorial and stenographic assistance. Finally, grateful acknowledgment is made to the Geological Survey of Canada for financial support of the basic research from which this contribution has stemmed.

Present Geological Status of the Toronto Formation

The deposits of the Toronto formation hold great interest in many respects. They contain the most complete organic record of warm interglacial conditions so far studied on this continent. Their stratigraphical position is almost as little known as it was when the deposits were first studied. The section is important because of its climatic implications. The flora and fauna of the interglacial beds indicate extensive deglaciation. Using R. F. Flint's words, "the Toronto formation is the most remarkable interglacial sequence yet uncovered in North America" (Flint 1949, p. 285).

Studies of the Toronto formation started about a hundred years ago and the authors refer to A. P. Coleman's papers (1933, 1941) for historical background of the subject. The problem has also been discussed by Flint (1949), Gray (1949), and Watt (1953, 1957).

The Pleistocene deposits in the Toronto region are underlain by the sedimentary rocks of the Palaeozoic. This entire foundation dips slightly towards the south. The bedrock is often exposed with its overburden of Pleistocene deposits in the several quarries and cuts in and near Toronto. Deposits of the Toronto formation are best exposed at the historically marked sites—the Don Valley Brickyard and the Scarborough Bluffs along Lake Ontario.

Flint (1949) summarized the stratigraphical and lithological features of the sequence constituting the Toronto formation, making use of the information supplied in Coleman's studies (1933). From this, the following composite Pleistocene section was constructed:

1. Lake Iroquois features (wave-cut cliffs, gravel bars, and finer sediments with fossils).
2. Upper till (sandy till with swell and swale topography veneering the older deposits).
3. Interglacial sand, possibly marine.
4. Middle till complex consisting of tills, interbedded with varved fine sediments.
5. Toronto formation (interglacial)
   - Scarborough beds: stratified clay, silt, and sand with peat.
   - —Probable conformity—
   - Don beds: lacustrine clay, sand, and gravel; peat.
6. Lower till (2 to 3 ft thick; fresh, no weathering of the upper surface).

Lithological comparisons have been made on exposures in the Don Valley Brickyard and at the Scarborough Bluffs. These observations by the junior author showed that the sand between the upper till and the middle till complex should be designated as interstadial. No evidence was discovered to indicate a marine origin of this sand. No peat layers or lenses, but only finely disseminated plant detritus in silt, sand, and clay were found in the Don and the Scarborough members of the Toronto formation.

The probable conformity between the Don and the Scarborough beds was questioned on basis of lithological evidence. Stratigraphical correlation of the Scarborough beds exposed in the Don Valley Brickyard and in the Scarborough Bluffs has been accepted in the present study on basis of Coleman's evidence. The account by Watt (1953, 1957) has established that new evidence integrating the events and lithology of the Toronto formation is rapidly accumulating. The junior author, with others, is shortly to publish data augmenting those already presented in an earlier account (Terasmae 1955).

An observation with some significance has arisen relating to the stratigraphical interpretation of the present study. It bears on the events following the deposition of the Scarborough beds. In one location at Scarborough, at the "Dutch Church", examination of deposits indicates a period of erosion following the deposition of the Scarborough beds. This was noted by Coleman (1933), who concluded that deglaciation was taking place at the time. On the other hand Watt (1953) suggested that the glacier was in fact advancing and that erosion was because of gouging. The present study suggests that at the Scarborough Bluffs this particular valley was cut during the nonglacial interval and subsequently filled with proglacial and glacial deposits.

Analysis of Plant Microfossils

The first attempts to isolate pollen, spores, and other microscopic remains from their mineral matrix were made by the senior author on samples taken from the Toronto Rapid Transit Subway excavation (Legget and Schriever 1958). Initially, botanical interpretation of this material was confined to the fibrous macroscopic fragments that prevailed in the unconsolidated sand samples. It was after it became clear that conclusive identification of many of these fragments would require considerable time that the emphasis was switched to examination of pollen and spores which were appearing in some of the preparations. Their definitive nature was conducive to quick and precise identification. Further, it was thought that they might have paleoecological significance, especially if they occurred in considerable number and demonstrated a frequency differential.

Eventually, large numbers of pollen and spores were isolated from these samples. They were identified and utilized for age determination of the stratum from which they were derived. Preliminary advice on these results was sent to A. K. Watt, Ontario Department of Mines, who supplied some of the samples. These results on age were later confirmed in the Muskeg Laboratory at McMaster University but at this time it was realized that they would be

of greater significance if compared with microfossil evidence from other samples of deposits of the Toronto formation. To this end extensive sampling was done on freshly cleared exposures chiefly at the Scarborough Bluffs, and at the Don Valley Brickyard.

Isolation of microfossils from the new sources was beset with fundamental difficulties; some occurred because of inadequate technique, others were imposed by inherent circumstances. The foremost difficulties encountered were the paucity of microfossils in the deposits and the possibilities of error, such as secondary deposition, differential destruction, and floating ability of various plant microfossils.

Some difficulties were anticipated. The first mentioned was accepted at the outset as a primary challenge in the project. Samples highly charged with organic debris like that provided from the Toronto Rapid Transit Subway excavation were almost nonexistent. Most of the samples were inorganic and it was questionable that they contained any organic entities. If they did, the organic matter was apparently so widely dispersed as to escape any attempt at detection.

Preliminary analysis confirmed this. Attention therefore turned to the problem of isolating relatively widely dispersed pollen grains and spores and at the same time concentrating them from the inorganic samples of somewhat larger volume. To achieve this, samples ranging in size from 200 to 500 cc were first dispersed in water. Sand was removed by multiple washing and decanting. Hydrochloric acid and hydrofluoric acid were subsequently applied to the residue to remove the carbonates and the silicious fraction of the sample. Coarse plant debris, if present, was removed by sieving and the treatment was ended by the acetolysis method to bring about further concentration of microfossils and give them a convenient yellow-brown color. Staining was not necessary. All these individual treatments have been described in detail by the junior author (Terasmae 1955).

With this treatment, microfossils in significant numbers were isolated for interpretation. There were still exceptions, but these were no longer attributable to deficiencies in technique. In such cases it was assumed that either no microfossils had been preserved, or, if they occurred, they were so infrequent as to be relatively ineffectual as significant indices except by reason of their relative paucity or perhaps absence. The different possibilities of error were given due attention during the interpretive stage of the study and the reliability of the results has been discussed elsewhere (Terasmae 1955).

The method and usefulness of plant and animal microfossils for describing and identifying Pleistocene interglacial and interstadial intervals has been convincingly outlined by Van der Vlerk (1955). In the present work too, allowing for all possibilities of error and other limitations, the major, long-time changes in climate and ecological conditions reflected in the microfossil assemblages show up with sufficient distinctiveness (cf. Table I) to provide the investigator with a 'tool' that can be efficiently used for paleoecological and stratigraphical interpretations. On this basis, correlation can be worked out between beds of the same age in different exposures.

This does not render less useful the plant macrofossils used by Coleman for working out a representative fossil plant assemblage for the sequence of the Don beds. This collection was qualitative. The study of plant microfossils, as disclosed in the present work, has some significant advantages over the study of macrofossils (Radforth and Rouse 1954; Terasmae 1955). The microfossils are produced in much greater numbers than macrofossils and are subsequently distributed much more uniformly over wide areas and deposited in representative assemblages in various kinds of sediments. They are found in sufficient numbers in relatively small samples. Analysis of plant microfossils is quantitative as well as qualitative in its nature. It is possible to calculate the relative abundance of each component in the past vegetation associations. It is possible to follow step by step the changes in plant microfossil assemblages from bottom to top of a sediment sequence by taking samples at close intervals. No doubt, the best results will be obtained when both macrofossils and microfossils are studied, because some plants produce very little pollen and it would seem that other plants have a very small chance to be represented by their microscopic remains in sedimentary deposits.

Now the writers turn to consideration of kinds, frequencies, distribution, and relationships of the microfossils revealed in the investigation.

A Comparison of Selected Plant Microfossil Assemblages from the Don Beds and the Scarborough Beds

Only a few selected plant microfossil spectra of fundamental significance will be presented in this paper. Subsequent to the study here reported, new and better exposures, particularly from the bottom sequence of the Don beds and the contact between the Don and the Scarborough beds, were uncovered. A detailed study was made of these exposures and samples were collected at close intervals for lithological and plant microfossil studies. Results of this later investigation will be published as soon as they are available.

The spectra selected for the present comparison are six in number and for convenience they are shown together in the six columns of Table I. The first spectrum was derived from a sample of the basal thin layer of clay (1 to 3 in. thick) of the Don beds, immediately overlying the glacial till on the Ordovician shale. This assemblage, supported by others from beds immediately overlying the basal clay, definitely proves that a hiatus of unknown length of time is present between the basal till and the bottom sediments of the Don beds, because the microfossil assemblage indicates already temperate conditions in clay overlying glacial till.

The second assemblage (Table I, column 2) was derived from the lower middle part of the Don beds. Most interesting is the presence of *Liquidambar* pollen in this sample. The pollen grains were compared with photographs and descriptions given by Traverse (1955) and there seems to be little doubt about the identification. A few pollen grains, reckoned among pollen of *Ulmus*, were of the *Planera* type. Positive identification, however, is still wanting.

TABLE I

Pollen and spore assemblages from the interglacial deposits

	Don beds			Scarborough beds		
	Basal clay 1–3 in.	Lower mid	Top	Lower	Mid	Subway beds
Arboreal pollen or spores						
Picea	1	—	—	—	25	—
Picea glauca	—	2	20	19	—	17
Picea mariana	—	2	15	15	—	6
Pinus	—	—	—	—	24	—
Pinus strobus	11	3	1	—	—	61
Pinus banksiana		8	28	55	—	
Abies	—	1	1	—	—	3
Juniperoid	8	—	—	—	—	3
Tsuga canadensis	—	4	—	—	—	—
Larix	—	—	1	—	—	5
Betula	1	2	1	13	4	ab
Alnus	1	—	3	16	—	2
Ulmus	25	8	1	—	—	—
Quercus	47	20	5	2	—	—
Acer	4	11	1	—	—	—
Fraxinus	—	2	—	—	—	—
Carya	20	4	1	—	—	1
Tilia	4	13	+	—	1	1
Fagus	—	18	—	1	—	—
Juglans	—	—	1	—	—	—
Carpinus	—	1	3	2	—	—
Salix	2	2	1	—	—	—
Eleagnus	—	—	—	1	—	—
Populus	cf. 1	—	1	—	—	cf. 6
Liquidambar	—	7	1 (derived)	—	—	—
Ilex type	—	—	2	2	—	—
Corylus	—	1	—	—	1	—
Non-arboreal pollen or spores						
Caryophyllaceae	—	—	—	—	—	1
Chenopodiaceae	3	—	—	1	—	—
Compositae	3	2	—	1	—	2
Cyperaceae	1	—	—	—	1	3
Ericaceae	—	—	—	1	2	13
Gramineae	5	—	3	—	1	13
Artemisia	—	—	1	2	—	—
Ambrosia	2	1	—	—	—	—
Sparganium	11	—	2	—	—	—
Nuphar	—	—	1	—	—	—
Cf. *Potamogeton*	1	—	—	—	—	—
Nymphaea	—	—	—	—	1	—
Osmunda	1	1	—	—	—	—
Equisetum	—	—	—	—	—	4
Ferns	2	7	5	—	1	6
Lycopodium	—	1	—	3	1	—
Selaginella	—	—	1	—	—	—
Lycopodium annotinum	—	—	+	3	—	—
Sphagnum	—	—	—	+	+	+
Unidentified NAP	13	6	5	1	6	2
Fungi	—	+	+	+	—	+

When the macrofossil assemblage, worked out by Coleman (1933), is considered together with the microfossil evidence, there seems to be little doubt that climate during deposition of the Don beds reached a thermal maximum, warmer than the present climate in the Toronto area. An annual mean temperature about five Fahrenheit degrees higher than the present seems probable. Coleman found leaf fossils of trees including *Asimina triloba*, *Maclura pomifera*, *Robinia pseudacacia*, *Quercus stellata* and *muhlenbergii*, *Fraxinus quadrangulata*, and *Chamaecyparis thyoides* in the Don beds. The northern distribution limit of these plants is well south of the Toronto area at the present time. For the complete list of macrofossils see Coleman (1933). The presence of extinct species of maple (*Acer pleistocenicum* and *Acer torontoniensis*) based on fossil leaves found in the Don beds was later proved erroneous by R. W. Brown (1942). These leaves were shown to belong to the sycamore (*Platanus*).

The third assemblage (Table I, column 3) came from the top part of the Don beds. Examination of it clearly suggests that cooler climatic conditions prevailed towards the end of deposition of the Don complex. The spectrum is primarily coniferous and the pollen from deciduous trees has markedly declined. The presence of another possible hiatus between the Don and the Scarborough beds will be discussed in connection with results from further investigations.

The fourth assemblage (Table I, column 4) was derived from sediments of the lower Scarborough sequence. This pollen spectrum can be readily compared with those from the Canadian boreal forest region.

The fifth assemblage (Table I, column 5) came from a sample of the middle part of the Scarborough sequence.

Finally, the sixth assemblage was derived from a representative sample of sand and plant debris collected from the Toronto Rapid Transit Subway excavation (Table I, column 6). This spectrum, dominated by pollen of coniferous trees, cannot be related to any known assemblage of plant microfossils from the Don beds but it can be compared readily with assemblages from the Scarborough beds. It is suggested that the sands, represented by the above sample, correlate with the sands of the Scarborough member of the Toronto formation. This conclusion is also supported by stratigraphical evidence.

Discussion

While procuring the evidence shown in the aggregate of Table I, consideration was given to the microfossil content of a number of sets of thinly laminated sediments. Analysis of these showed that in some cases all laminae were devoid of microfossils except for a few of the layers of the series, whereas in other cases fossil content was marked in nearly all laminae. This contrast was a characteristic phenomenon and it afforded stratigraphical and interpretive evaluation which will be referred to briefly in discussion along with other evidence and conclusions that have arisen.

That plant microfossils did, in fact, occur generally and in abundance in the mineral matrix is stratigraphically useful in three respects. In the first

place, where all of the thin laminae were found charged with microfossils at all or most levels, it was highly suggestive that they were not varves. This proved helpful in diagnosing for secondary geomorphological relationships in exposures. Some series, on the other hand, were relatively barren. When their laminae showed consistent gradational alternating between coarse and fine sediments it seemed reasonable to conclude that they were varves; where microfossils did occur (usually in the coarse-grained components) they were few and sporadic, thus lacking interpretive significance. Deposition was probably close to the edge of a glacier lobe of sufficient magnitude to effect continuously cold conditions, thus discouraging plant growth in the area. The sediments that simulated varves and which were richly supplied with microfossils probably signified local fluvial sedimentation not necessarily a function of season. It is hoped for the future that such contrasting circumstances of deposition can be applied to interpret local climatic effects, direction of movement of the glacial front, and patterns of erosion.

A second circumstance with stratigraphical implication is provided in the disclosure of the Toronto Rapid Transit Subway excavation assemblage of microfossils. Not only is it possible to draw conclusions on age relations as a result of the analysis, but also it now becomes reasonable to infer that other samples of sediments isolated from sequences of unknown relations can be given stratigraphical significance through microfossil examination. Geographically widely separated sequences should also be easier to correlate without the necessity of placing undue emphasis on lithological interpretation.

The major aid to stratigraphical interpretation is the large-scale implication of the last-mentioned principle. This supplies the third category of assistance that the microfossil study affords. Although it is not the intention of the authors to claim that microfossil comparisons such as those shown in Table I are in themselves conclusive as a means of establishing the age relations for the Toronto formation in the Toronto area, they are nevertheless a fundamental requirement.

That the Don beds belong to the Sangamon interglacial interval now appears certain. This has its local significance, but in addition it facilitates interpretation for widely separated areas. For example, when the senior author attempted to designate the age of certain buried peat in the Lake St. John area of Quebec, a comparative warm climate flora was not known. The present work has lessened the cause for hesitation on a warm climate designation for the Lake St. John location. Further evidence supporting the view that widespread correlation for Eastern Canada is now possible has been disclosed by the junior author and will be published shortly.

Although there is little to distinguish the upper Don beds from the Scarborough sequence, on basis of microfossil contrast there is some difference of significance. The sporadic pollens of deciduous trees in the upper Don beds are more numerous than in the Scarborough beds. A botanical hiatus therefore accompanies a stratigraphical one. On a botanical basis, a claim that the Scarborough beds are Wisconsin in age might in some measure be reasoned.

However, the upper Scarborough beds yielded a more subarctic flora, as evidenced through comparative lack of deciduous representation, than the lower Scarborough sediments (cf. Table I, columns 4 and 5). Indeed, there is mutual support in the botanical–geological evidence that the Scarborough beds are pre-Wisconsin in age.

Paleoecological evidence largely relates to climate and change in emphasis on relative coniferous–deciduous predominance throughout the history of the Don and Scarborough beds. Until more evidence is at hand, enough has been already concluded or inferred on this aspect of the investigation.

No analysis of local floral constitution and change has been attempted, and it is on this topic that brief preliminary deduction may be permitted. The sample from the Toronto Rapid Transit Subway excavation as a particular example revealed one ecological category. It was somewhat upland, emphasized by Gramineae and Ericaceae with Cyperaceae less predominant, with pteridophytic intruders, and with only negligible representation from Compositae. This group of types, along with the high incidence of *Picea*, reconstructs a landscape singularly like that covering much of our muskeg in the North (Radforth 1952). The cyperaceous component would be in all likelyhood *Eriophorum* spp. which characteristically interrupts the wooded areas to which the ericaceous members are largely confined. This kind of landscape is traversed by confined and somewhat tortuous fluvial action and seldom are the deposits of organic material thick. Pockets where detritus resembling the sample can gather are frequent as small shallow open ponds. Such assemblages of cover types are now found south of the Hudson Bay coast. Occasional mixtures of *Populus* and *Pinus banksiana* are also to be expected on crowns of well-drained sites.

Reconstruction of interglacial landscape is thus becoming a possibility on a basis that depicts morphological character in the groupings of plants that the microfossils represent. At the moment, however, the inference is chiefly speculative and the idea remains as a reasoned hypothesis.

References

ANDERSEN, S. TH. 1954. A late-glacial pollen diagram from southern Michigan, U.S.A. Danmarks Geol. Undersøgelse II, No. 80.

BROWN, R. W. 1942. Supposed extinct maples. Science, **96**, 15.

COLEMAN, A. P. 1933. The Pleistocene of the Toronto region. Ontario Dept. Mines Ann. Rept. (1932), **41**, Pt. 7, 1–55.

——— 1941. The last million years. A history of the Pleistocene in North America. Univ. Toronto Press, Toronto, Ontario.

FLINT, R. F. 1949. Glacial geology and the Pleistocene epoch. John Wiley & Sons, Inc., New York.

GRAY, A. B. 1949. Sedimentary facies of the Don member of the Toronto formation. Masters Thesis, University of Toronto, Toronto, Ontario.

LEGGET, R. F. and SCHRIEVER, W. R. 1958. Soil studies for Canada's first subway. In press.

HALLIDAY, W. E. D. 1937. A forest classification for Canada. Canada Dept. Mines Resources, Lands, Parks, Forests Branch, Bull. No. 89, 1–50.

HINDE, G. J. 1878. Glacial and interglacial strata of Scarborough Heights. J. Can. Inst. (Royal Canadian Institute).

Native Trees of Canada. 1950. Can. Dept. Resources Develop., Forestry Branch, Bull. No. 61.

RADFORTH, N. W. 1952. Suggested classification of muskeg for the engineer. Eng. J. **35**, 1199–1210.

RADFORTH, N. W. and ROUSE, G. E. 1954. The classification of recently discovered Cretaceous plant microfossils of potential importance to the stratigraphy of Western Canadian coals. Can. J. Botany, **32**, 187–201.

TERASMAE, J. 1955. A palynological study relating to the Toronto formation (Ontario) and the Pleistocene deposits in the St. Lawrence Lowland (Quebec). Ph.D. Thesis, McMaster University, Hamilton, Ontario.

TRAVERSE, A. 1955. Pollen analysis of the Brandon lignite of Vermont. U.S. Bureau Mines, Rept. Invest. No. 5151.

VAN DER VLERK, I. M. 1955. The significance of interglacials for the stratigraphy of the Pleistocene. Quarternaria II, 35–43.

WATT, A. K. 1953. Glacial geology of the Toronto–Orangeville area, Ontario. Guide book for field trip No. 3; Annual meeting of the Geol. Soc. America in Toronto.

——— 1957. Pleistocene geology and ground-water resources of the township of North York, York County. Ontario Dept. Mines Ann. Rept. (1955), **64**, Pt. 7.

SUCROSE SYNTHESIS IN THE SEEDLING WHEAT LEAF[1]

G. M. WARD

Abstract

Glucose supplied as a metabolic substrate to excised wheat leaves by means of the vacuum infiltration technique is converted to sucrose. It has been shown that the rate of this conversion is not wholly referable to enzyme kinetics for the reaction is a complex physiological process. It is conditioned by numerous factors, being stimulated by high concentrations of substrate, by low temperature during incubation, by both high and low relative humidity, and being depressed by starvation. A new technique of first injecting and then withdrawing the substrate has indicated that the rate of cellular absorption of the substrate can be a limiting factor in the synthesis of sucrose.

Introduction

For a number of years it has been known that excised leaves, fed with solutions of glucose or other hexoses, will synthesize sucrose in the absence of light. This conversion has been demonstrated with three varieties of technique, i.e. floating leaves on sugar solutions, standing leaves with their cut ends in sugar solutions, and the vacuum infiltration procedure. Nelson and Auchincloss (13) showed sucrose to be synthesized in potato tissue from glucose or fructose. Virtanen and Nordlund (16) found this conversion to occur in red clover and wheat, Hartt (6) obtained similar results with sugar cane leaves, and McCready and Hassid (12) investigated sucrose synthesis in barley shoots. In 1947 Krotkov (9) drew attention to the fact that Russian workers had been using the vacuum infiltration technique in metabolism studies since 1936; he described some of their results dealing with sugar metabolism in a wide variety of tissues and plant species. Prior to this date, however, the vacuum infiltration procedure was thoroughly investigated by S. P. Hawkey in the laboratory of F. F. Blackman at Cambridge (7) although the results have never been published.

This paper is the report of an investigation of the synthesis of sucrose in the excised seedling wheat leaf from specific substrates supplied by the vacuum infiltration technique. Two previous papers (17, 18) have dealt with other phases of sucrose and its metabolism in the wheat leaf.

Materials and Methods

The wheat leaf material used in this research was of the same variety as that grown previously (17). It was grown in soil in the uniform, waxed tins under the same artificial conditions described by Duff and Forward (4). In all cases, except where indicated otherwise, mature 13-day leaves were used as experimental material. Individual samples consisted of 25 uniform leaves.

[1]Manuscript received March 9, 1960.
Scientific Contribution No. 59, Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

Determinations of reducing sugars and sucrose were made upon cleared alcoholic extracts of fresh leaf tissue by the Hanes modification (5) of the Hagedorn and Jensen ferricyanide method. The inversion of sucrose was carried out with acid.

A schedule of procedure was adopted which has been used as a standard of comparison throughout the investigation. Briefly, this schedule may be summarized thus: a 25-leaf sample of uniform standard leaves was harvested, starved in the dark for 24 hours, weighed, vacuum-infiltrated with 5% glucose at 25° C, reweighed, incubated for 22 hours at 22.5° C and 55% relative humidity, then analyzed for reducing sugars and sucrose.

The darkening or starvation of leaves prior to infiltration has become a standard practice with most workers employing this technique for carbohydrate studies. It was found in this investigation that the darkening experience decreases the amounts of both reducing and non-reducing sugars in the leaf but neither fraction is entirely eliminated even with prolonged darkening. These results are not in agreement with those of McCready and Hassid (12), who claim that sucrose completely disappears from barley seedling tissue after a dark experience of 24 hours and that reducing sugars do not start to decline in percentage until the sucrose is fully depleted. Roberts (15) reports considerably lower values for sucrose in darkened leaves than those reported here.

The analytical results are reported in terms of milligrams of sugar per gram of original fresh weight of leaf tissue. Some investigators have reported sucrose synthesis in terms of a time-rate. Kursanov (11) and Krotkov and Bennett (10) used this method of expression. It is considered, however, that this does not give an adequate representation of what occurs in the tissue. Since respiration is a continuous factor throughout the experiment, the supply of sucrose is being constantly depleted so that the final measurement of sucrose in the incubated leaves is the resultant of a number of reactions which may be stated as follows:

sucrose present in illuminated leaf at isolation,
minus sucrose respired during starvation,
plus sucrose synthesized during incubation,
minus sucrose respired during incubation and handling.

In all experiments the amount of handling was reduced to a mininum and carried out in the shortest possible time in order to minimize interference due to stimulation respiration (14). The customary procedure for arriving at a true value for sucrose synthesis in leaves infiltrated with glucose is to infiltrate a corresponding sample of leaves with distilled water and subject it otherwise to the same treatment given to the glucose-infiltrated sample. This blank determination was carried out in every experiment of this investigation. The difference in sucrose content between these two samples is usually regarded as representing the amount of sucrose synthesized from the infiltrated metabolite. It is possible, however, that the presence of such a large supply of respiratory substrate may stimulate respiration in the glucose-infiltrated sample to such

PLATE I

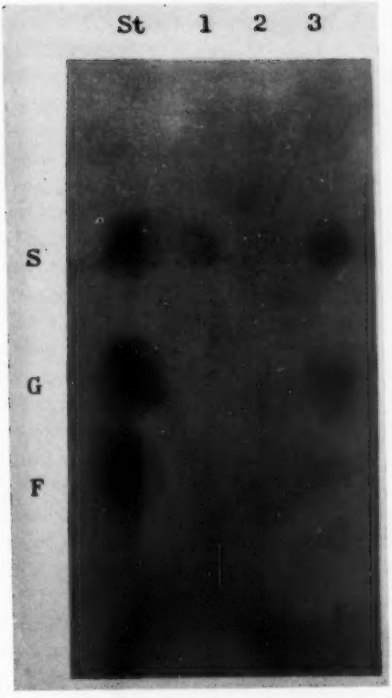


FIG. 1. Chromatogram of sugars in extracts of infiltrated wheat leaves.
St, standard; S, sucrose; G, glucose; F, fructose.
1, Illuminated wheat leaf; 2, starved wheat leaf; 3, glucose-infiltrated wheat leaf.

an extent that in respect to sugar loss the two samples will not be strictly comparable. Furthermore, the factor of hydration strongly influences metabolic balances such as those between protein and amino acids and between starch and sucrose. Therefore, caution must be used in the interpretation of results obtained by the employment of water-injected controls.

Another factor involved in the transformation of infiltrated metabolites is the absorption of the metabolite from the intercellular space into the cell prior to the transformation. In this investigation a technique has been developed for determining the amount of absorption in reference to the synthesis that occurs. The procedure is explained in detail in a following section, but it should be mentioned here that the capacity of a leaf to transform glucose into sucrose cannot be expressed in terms of a time-rate without taking into account this factor of cellular absorption. No previous attempt has been made to estimate the magnitude of this factor although Krotkov and Bennett (10) recognize that it may be involved.

In practice it was found that a water-infiltrated sample incubated under the standard conditions for 22 hours contained about 1.50 mg sucrose per gram of fresh tissue while a corresponding sample infiltrated with 5% glucose and similarly incubated contained about 5.50 mg sucrose per gram of fresh tissue. The slightest variation in technique particularly during infiltration caused considerable departure from these mean values.

Taking all these factors into consideration, it was decided that an adequate representation of sucrose synthesis could be obtained by refraining from the use of a time-rate expression of results and by using the value for sucrose content of the glucose-infiltrated leaf without subtracting the value for the water blank.

The infiltration technique involves a chronological sequence of events which takes place while the leaf is still an actively metabolizing tissue. The environmental conditions that exist at each stage of the process have an important effect upon the amount of sucrose synthesis that occurs and upon the measured amount of sucrose in the sample when it is finally analyzed. The study of the effects of these conditions has constituted the main body of the research.

The identity of the product synthesized from infiltrated hexoses has been discussed by both McCready and Hassid (12) and Krotkov and Bennett (10). The fact that sucrose is the synthesized sugar has been confirmed by Bidwell (1), who made a chromatographic analysis of extracts of infiltrated wheat leaves.

It was considered necessary to repeat this confirmation in the present investigation. One sample of starved leaves was infiltrated with water, a second with a 5% solution of glucose. Both samples were allowed to incubate for 22 hours, then were extracted with alcohol. The extract was freed of alcohol, cleared, and then cautiously evaporated to a small volume to concentrate the sugars present. A chromatographic separation was then carried out on both extracts by the procedure previously described (17). Column 2 in Fig. 1 represents the result obtained by chromatographing an extract of

starved wheat leaves. It is not possible to identify any sugars in this column, which indicates that starvation has effectively depleted the leaves of any sugars detectable by this technique. Column 3 shows the separation obtained in the sample of starved wheat leaves infiltrated with glucose. The very definite sucrose spot establishes beyond doubt the identity of the synthesized sugar. The residual glucose in the wheat leaf is apparent, also a faint indication of a small quantity of fructose. This evidence together with the proof of the existence of native sucrose in the wheat leaf advanced in a previous paper (17) is considered to be a sufficient indication that sucrose is the actual product of synthesis.

Experiments and Results

Effect of Incubation Time

The effect of the duration of incubation upon the amount of sucrose synthesis was tested in two experiments. In the first the leaves were harvested prior to starvation, in the second they were starved before harvesting. The results of these two tests are shown in Fig. 2. It will be observed that there is a certain period during which the net amount of sucrose in the leaf increases but thereafter it decreases. In considering the evidence on the problem of incubation time it is necessary to decide whether a short or a long incubation will be employed as a standard procedure. A short incubation would require a time progress definition and the estimation of an initial rate. Such a procedure is too complex and too refined for the variable physiological mechanisms involved in this transformation. If a long period of incubation is used, the question arises—how long should the period be? The data presented here indicate that it should be not longer than 22 hours. This agrees well with the conclusions of McCready and Hassid (12), who selected a similar period of incubation for their infiltration experiments with barley.

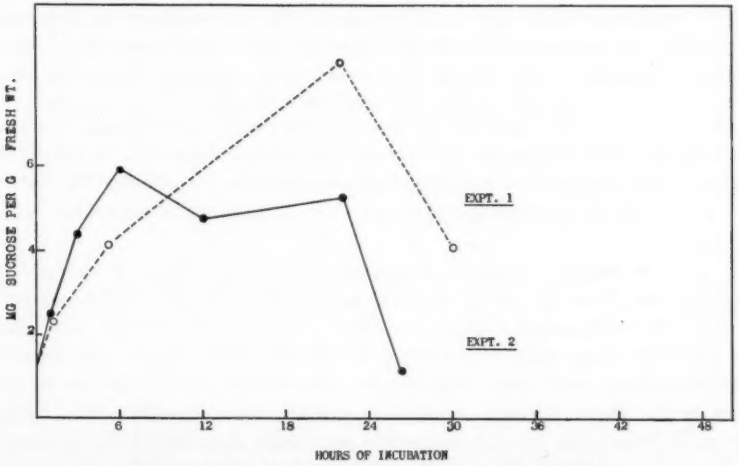


FIG. 2. Effect of incubation time upon sucrose synthesis.

Effect of Substrate Concentration

The effect upon sucrose synthesis of various concentrations of infiltrated substrate was tested in two experiments. One experiment was done with leaves harvested prior to starvation and another with leaves harvested after starvation. The amount of synthesis occurring in 22 hours increased with increasing concentration of infiltrated glucose solution up to concentrations of 25% as indicated in Fig. 3. When concentrations of 10% and above were used, the leaves were found to be in a flaccid condition after infiltration probably due to plasmolysis, but they were not seriously injured and appeared to recover during the incubation period. In any case the process of sucrose synthesis continued to operate.

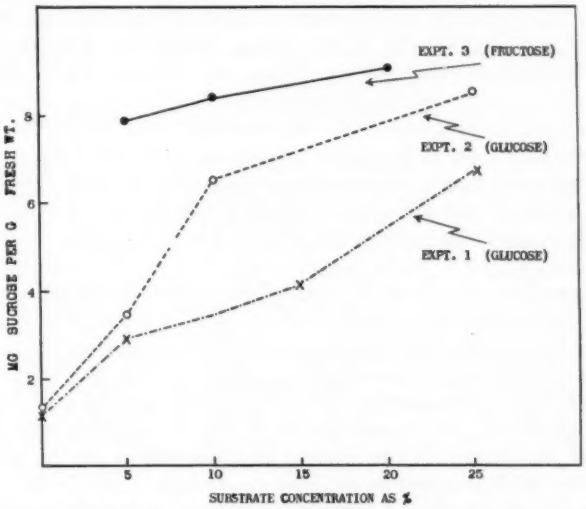


FIG. 3. Effect of substrate concentration upon sucrose synthesis.

Additional samples were infiltrated with three concentrations of fructose and a similar trend was observed (Fig. 3) but the amount of synthesis was higher than when glucose was employed as the substrate. This result was confirmed on three other occasions when fructose was infiltrated, but it does not agree with the findings of Krotkov and Bennett (10), who state that sucrose synthesis proceeds equally well from either a mixture of glucose plus fructose or from glucose or fructose infiltrated singly. It will be observed that the graph in Fig. 3 is plotted as a substrate concentration – sucrose relationship which thus ignores the actual amounts of infiltrated sugars. However, examination of the weights of infiltrated liquid revealed that the amounts infiltrated were actually greater for glucose than for fructose. Hence the conclusion is justified that fructose is a more facile substrate for sucrose synthesis. The results obtained in this experiment do not represent a true substrate-concentration effect since the graphs in Fig. 3 do not have the

hyperbolic form characteristic of enzyme systems in which concentration of substrate exerts its full kinetic effect. The increase in synthesis is certainly due to an increasing supply of available substrate, but the final concentration of sucrose measured in each instance represents the net result of several processes such as synthesis, variable respiration, and the rate of penetration of substrate into the cells.

The effect of concentration having been determined, a 5% glucose solution was adopted as the standard infiltration medium for the balance of the investigation. This represents a useful working concentration that results in measurable amounts of synthesis and has no ill effects upon the tissue.

Effect of Relative Humidity during Incubation

Early in this research it was observed that the relative humidity of the atmosphere surrounding the leaves during incubation was an important factor in the sucrose synthesis transformation. Kursanov (11) investigated this point and found a very definite effect over a certain range of "water loss". In the present investigation two experiments were conducted. Artificial atmospheres with a series of relative humidities from 0 to 100% were set up in glass jars using varying concentrations of sulphuric acid. The volume of acid used in each jar was sufficient to absorb any water lost from the incubating tissue without seriously altering the relative humidity of the atmosphere. A relative humidity value was estimated for each jar. The atmospheres were allowed several hours to come to equilibrium at the incubation temperature. Infiltrated leaves were then introduced into each jar where they were allowed to incubate in the dark for the usual period of 22 hours before analysis. In the first experiment the leaves were standing in beakers with their ends in distilled water. Each beaker was covered with filter paper and the leaves protruded through a small hole. No measurements of relative humidity were made but from visual observation of the amount of condensation on the walls of the jars, which varied from heavy in the presence of water to none in the presence of concentrated sulphuric acid, it was apparent that a real gradation of moisture conditions existed throughout the series. Chemical analysis revealed almost no variation in the sugar content of these samples, indicating that the various atmospheres had no effect upon sucrose synthesis in this instance. In appearance the leaves of all samples were quite similar, being dark green and turgid. The conclusion is that as long as the excised leaves are supplied with water through their cut ends, the synthesis process is apparently independent of the relative humidity of the surrounding atmosphere.

In the second experiment the above procedure was repeated with one change, namely, the samples of leaves were not standing in water during the incubation period. This change had an important effect upon the synthesis of sucrose. Results of the final experiment are presented in Fig. 4.

The analytical data for sucrose synthesis in these experiments are expressed in terms of milligrams of sucrose per gram of original fresh weight of tissue. Therefore the changes shown in Fig. 4 are not influenced by differences in the current fresh weight of the tissue.

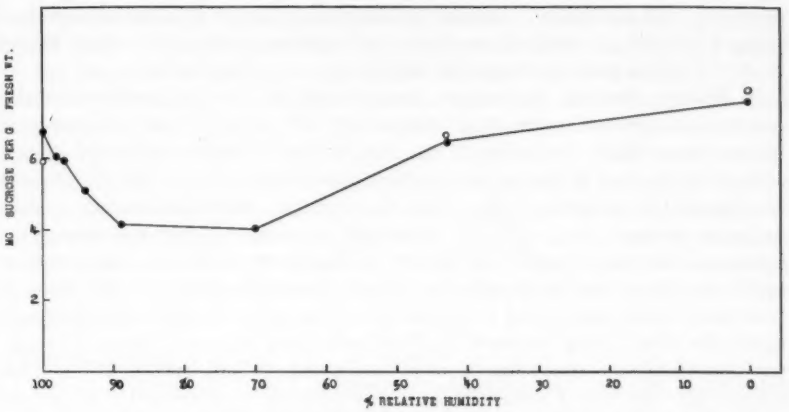


FIG. 4. Effect of incubation humidity upon sucrose synthesis.

Leaves which were exposed to relative humidities of 70% to 100% showed no visible signs of the progressive dehydration which was occurring. The dehydration of these leaves is obviously reversible and it induces sucrose synthesis. It can be compared with the reversible starch synthesis induced by hydration in the starch $\rightleftharpoons$ sucrose synthesis. This transformation is complicated, reversible, and strictly physiological. On the other hand leaves subjected to relative humidities of 70% to 0% show visible signs of a progressive desiccation which is irreversible and which also induces sucrose synthesis. This, however, is non-physiological. A similar type of study was carried out by Kursanov as quoted by Krotkov (9), but the conditions of desiccation differed from those used in this experiment and no increased synthesis with increasing desiccation was reported.

Effects of Temperature

The effects of temperature upon in vivo sugar transformations are even more pronounced than the effects of humidity. The capacity of the leaf for sucrose synthesis may be influenced by the temperature of the atmosphere surrounding the leaves during growth, during starvation, or during incubation. Several different combinations of temperature were tested in four experiments. The results of these are summarized in Table I. The data presented indicate the following points:

1. A high temperature experience during incubation results in a lowered sucrose content. This is a complex effect. It is probably true that respiration is accelerated and thus the rate of sucrose depletion is increased. But another effect is also manifest here. Many years ago F. F. Blackman (2) investigated and reviewed the effects of high temperature on the vital processes of normal plant tissue, showing that with a gradual increase of temperature the more highly organized and complex processes such as growth and photosynthesis are the first to suffer impairment, while respiration and similar exothermic

reactions will continue to operate at somewhat higher temperatures before being inhibited. It would thus appear that sucrose synthesis in wheat leaves at 37° C suffers a certain degree of high-temperature inhibition.

2. With a lowered incubation temperature sucrose accumulates in the leaves to a greater extent than when they are incubated at the standard temperature. That this effect is not due to the liberation of bound sugars already in the leaf is proved by the data presented in Table I for the sucrose content of the water controls. It will be observed that these leaves contain no more sucrose than controls incubated at standard temperatures. This increased sucrose accumulation appears at first glance to be due to augmented synthesis. It is not reasonable to believe, however, than an endothermic reaction of this type would be promoted by lowering the temperature. Once again the effect being measured here is both complex and indirect. The respiration of 13-day wheat leaves at 7° C is about 40% of that at 22° C. This respiration loss could result in an accumulation of respiratory substrate which would account for a considerable proportion of the sucrose content. Another factor to be considered is increased oxygen availability at low temperature which has been shown by McCready and Hassid (12) to augment sucrose synthesis. Still other factors may be involved and the net result when low temperature acts on a complex pattern of reaction could readily be an accumulation of synthesized sucrose.

TABLE I

Effect of temperature on sucrose synthesis

Starvation temperature	Incubation temperature	Leaf analysis (mg/g fr. wt.) Reducing sugar	Sucrose
Experiment 1 (leaves starved after harvesting)			
22.5	22.5	14.79	3.14
22.5	27	8.86	2.65
22.5	37	8.73	2.14
Experiment 2 (leaves starved before harvesting)			
7	22.5	8.02	3.96
22.5	7	5.62	8.46
22.5	37	4.04	3.20
Experiment 3 (leaves starved before harvesting)			
7	22.5	15.65	6.30
22.5	7	7.15	9.19
7	7	11.30	9.02
Experiment 4 (leaves starved after harvesting)			
7	22.5	10.98	4.37
7	7	8.73	7.93
Controls			
7	22.5	3.70	1.49
22.5	7	2.86	.76
22.5	37	2.56	1.00

3. When the starvation temperature is lowered, the net amount of sucrose found after incubation is always less than in corresponding leaves starved at the standard temperature. This might appear to be evidence for impairment of the synthesis mechanism, but here again the respiration effects must be accounted for. Depletion of the original sucrose reserves by starvation is not as complete at the low temperature as at the standard temperature. This can be seen in the slightly higher value for sucrose in the control starved at low temperature. Subsequent respiration during incubation is not sufficient to reduce the sucrose reserves to the value found in leaves kept at standard temperatures throughout. However, the higher temperature of incubation depletes the sucrose reserves at a faster rate than in leaves incubated at low temperature.

Effect of Starvation

The starvation respiration of standard wheat leaves has been investigated and described by Duff and Forward (3, 4). The corresponding changes in sugar content of starving leaves have been described by Krotkov (8). Krotkov and Bennett (10) have added a description of the effect of starvation upon the sucrose synthesis capacity of infiltrated wheat leaves. The experiments

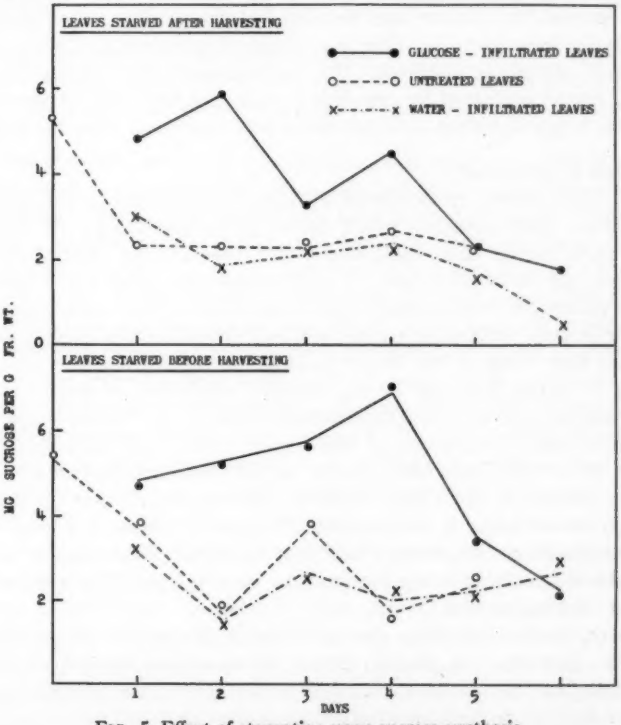


FIG. 5. Effect of starvation upon sucrose synthesis.

described in this section constitute a repetition and an extension of work of Krotkov and Bennett. Their experiments were done with excised leaves exclusively. In the present investigation starvation was carried out with both excised and attached leaves. Three series of analyses were made with both types. In the first the effect of starvation upon the sugar content of untreated leaves was tested; the second series was a check in which leaves were infiltrated with water, then incubated and analyzed. In the third series the effect of starvation upon sucrose synthesis from infiltrated glucose was tested. All leaves, both excised and attached, were first sampled after 12 hours of illumination, then were placed in the dark and samples were taken for treatment and testing daily for a period of 5 days. The results are given in Fig. 5.

It should be pointed out that the infiltrated leaves were subjected to an additional day of isolation because of the fact that they were subsequently incubated in the dark for 22 hours before analysis. This has been taken into account in plotting the analytical data in Fig. 5.

The process of simple starvation produced a more persistent decline in the sucrose content of excised leaves than in that of attached leaves. The sucrose synthesis capacity of starving excised leaves decreased in a somewhat erratic fashion. There was a slight rise on the third day which may correspond to results reported by Krotkov and Bennett but this effect was not very clearly defined with these samples. This is probably accounted for by the different methods of expressing synthesis. The sucrose synthesis capacity of starving attached leaves increased for the first 3 days and then fell off very rapidly. With both types of leaves autolytic breakdown occurred after the fifth day.

Penetration Experiments

When wheat leaves are infiltrated with various solutions as described in this investigation, they assume a much darker green appearance. Although they are still quite turgid, they seem heavy and more opaque. The uniformity of infiltration can easily be judged by visual inspection. Any spots or areas not properly infiltrated have a much lighter appearance. The dark-green appearance of uniformly infiltrated leaves persists throughout the incubation period, indicating that most of the liquid remains in the intercellular spaces. The quantity of liquid that can be successfully infiltrated is usually from one-third to one-quarter of the original weight of the leaves. Krotkov and Bennett (10) claimed that the presence of this liquid in the intercellular spaces might interfere with normal gas exchange to such an extent that sucrose synthesis would be depressed. They have therefore devised a technique for drying the infiltrated leaves prior to incubation. Whether or not such a depression of sucrose synthesis occurs, it was found possible in this investigation to observe appreciable fluctuations in synthesis under varying conditions without resorting to any drying process.

However, it was felt that the question of absorption of the infiltrated metabolite into the cell should receive serious consideration. It is highly improbable that the synthesis of sucrose occurs free in the intercellular spaces. It is more likely that the infiltrated glucose is first absorbed by the surrounding

cells or adsorbed on the protoplasmic surfaces and then transformed. Therefore it was thought that if the infiltrated liquid could be pulled out of the leaf again, some light would be thrown on the problem of absorption. A technique for centrifuging was devised which extracted all the infiltrated liquid and left the leaves practically undamaged. The procedure was as follows: A sample of 25 infiltrated leaves was firmly tied together at the tip ends with a small rubber band. The band was then securely fastened to a holed rubber stopper which was tightly fitted into a 50-ml conical centrifuge tube. The assembly was so arranged that the leaves were held loosely against the base of the stopper and were suspended well above the bottom of the tube. A perforated platinum disk was placed in the constricted end of the tube to prevent the leaves from stretching down into the bottom where the liquid gathered. The sample was then centrifuged at low speed for about 5 minutes. This was usually sufficient to remove all the infiltrated liquid. There was a slight amount of damage at the tips of the leaves where they were tied by the rubber band but this did not appear to interfere with the normal processes of respiration and synthesis, and after the usual period of incubation most of the visible signs of injury had disappeared. This liquid which was centrifuged out of the leaf was weighed, quantitatively transferred to a volumetric flask, and analyzed for reducing sugars and sucrose.

With the development of this technique it was now possible to determine the speed with which the infiltrated metabolite is absorbed into the cell and to determine the effect upon sucrose synthesis. Representative data are presented in Table II.

A sample of infiltrated leaves was centrifuged immediately after infiltration, then allowed to incubate for the normal period of 22 hours before analysis. Another sample was allowed to incubate for 6 hours before being centrifuged. It was then allowed to complete the 22 hours of incubation before analysis. A third sample was allowed to incubate for the full period of 22 hours before being centrifuged. In all instances both leaves and centrifuged liquid (centrifugate) were analyzed for sugars. Samples 6–11 in Table II represent two separate tests of this procedure. When the infiltrated liquid is immediately centrifuged out, then the leaf is deprived of substrate for conversion. However, simple centrifuging did not remove all of the infiltrated liquid and enough substrate remained to produce an increase in sucrose content. Furthermore, the concentration of the centrifuged liquid was less than that of the infiltrated liquid, which indicates that during the few minutes before immediate centrifuging could be carried out some glucose absorption had occurred providing substrate for sucrose synthesis. In sample 6 sucrose content went up to 4.68 mg per gram and in sample 9 to 3.66 mg per gram. Within 6 hours a good proportion of infiltrated glucose had been absorbed into the cells and most of it converted to sucrose. Sample 10 was not uniformly infiltrated and does not give an adequate picture of the leaf at that stage. In 22 hours almost all the glucose had been absorbed but it was not all converted to sucrose. There was a considerable accumulation within the cell as reducing sugar.

TABLE II

Analytical data from penetration experiments (expressed as mg/gh fres weight)

Sample No.	Centrifuging and incubation treatment	Infiltrated solution			Centrifuged solution				Leaf analysis	
		Type	Wt. of soln.	Calculated wt. of dissolved sugar	Wt. of soln.	Calculated original wt. of dissolved sugar	Analysis		Reducing sugar	Sucrose
							Reducing sugar	Sucrose		
1	Control, no centrifuging	Water	279						2.68	1.56
2	Control, no centrifuging	5% glucose	296	14.80					7.26	5.89
3	Centrifuge, incubate 22 hr	Water	312		221		.10	.00	2.56	1.34
4	Incubate 22 hr, centrifuge	Water	310		293		.07	.00	2.52	1.33
5	Incubate 22 hr, centrifuge	Water	209		182		.11	.00	2.51	1.50
6	Centrifuge, incubate 22 hr	5% glucose	281	14.05	222	11.10	3.59	.00	3.76	4.68
7	Incubate 6 hr, centrifuge, incubate 16 hr	5% glucose	298	14.90	289		1.57	.43	4.80	5.43
8	Incubate 22 hr, centrifuge	5% glucose	305	15.25	300		1.41	.45	7.28	5.26
9	Centrifuge, incubate 22 hr	5% glucose	294	14.70	254	12.70	7.64	—	4.11	3.66
10	Incubate 6 hr, centrifuge, incubate 16 hr	5% glucose	132	12.60	132		.29	.21	3.24	3.40
11	Incubate 22 hr, centrifuge	5% glucose	276	13.80	259		.27	.54	6.27	6.11
12	Infiltrate 5% glucose, centrifuge	5% glucose	317	15.85	263	13.15	8.19	—		
	Infiltrate water, centrifuge, incubate 22 hr	Water	290		290		2.52	.23	2.81	2.31
13	Incubate 22 hr, centrifuge	10% fructose	299	29.90	299		.80	.55	10.82	10.80

With some reservations as to the absolute reproducibility of the data in Table II, it is still possible to obtain from these figures some measure of the efficiency of the synthetic mechanism in the leaf for the utilization of supplied substrate. The following calculations will illustrate this point.

Sample 6

14·05 mg glucose infiltrated,
3·59 mg glucose recovered in centrifuged liquid,
10·46 mg glucose retained = 75% of that infiltrated.

5·26 mg sucrose found in leaves of sample 8,
1·33 mg sucrose found in leaves of sample 4,
3·93 mg sucrose, corrected value for leaves incubated 22 hours before centrifuging.

4·68 mg sucrose found in leaves of sample 6,
1·34 mg sucrose found in leaves of sample 3,
3·34 mg sucrose, corrected value for leaves centrifuged before incubation = 84% of corrected sucrose value in sample 8.

Sample 9

14·70 mg glucose infiltrated,
7·64 mg glucose recovered in centrifuged liquid,
7·06 mg glucose retained = 48% of that infiltrated.

6·11 mg sucrose found in leaves of sample 11,
1·33 mg sucrose found in leaves of sample 4,
4·78 mg sucrose, corrected value for leaves incubated 22 hours before centrifuging.

3·66 mg sucrose found in leaves of sample 9,
1·34 mg sucrose found in leaves of sample 3,
2·32 mg sucrose, corrected value for leaves centrifuged before incubation = 48% of corrected sucrose value in sample 11.

These calculations give some indication that the net synthesis of sucrose is related to the amount of glucose that is present inside the leaf.

When it became apparent that all the sugar solution was not extracted from the tissue by simple centrifuging, another modification was introduced. A sample was infiltrated with glucose solution, immediately centrifuged, then reinfiltrated with water, then centrifuged again, and finally allowed to incubate for the normal period and analyzed. Sample 12 in Table II shows the results of one such test. It will be seen that a large proportion of the infiltrated glucose was pulled out of the leaf in the first centrifuging but an appreciable increment also came out in the washing procedure which followed. In this

sample the amount of accumulated sucrose in the tissue at the end of the incubation period is very little more than that found in a water-infiltrated sample. It is assumed that in the few minutes required for handling the sample a small amount of substrate was absorbed by the cells.

Tests of the centrifuging technique were made upon water-infiltrated samples both before and after incubation. Results on samples 3, 4, and 5 indicate slight positive tests for reducing sugars but these are not considered to be significant. There was no evidence of sucrose here. The small values found for sucrose in several centrifugates in Table II may be due to conversion taking place in the intercellular spaces but it is more probable that they are caused by sucrose diffusing out of the cells into the infiltrated liquid.

Finally, a sample of leaves infiltrated with a 10% solution of fructose and allowed to incubate for 22 hours absorbed almost all the substrate and built up a high concentration of both sucrose and reducing sugars within the cells. It should be pointed out that when leaves that have not been infiltrated are subjected to centrifuging, no liquid is extracted from the tissue, thus indicating that the centrifugate represents infiltrated liquid and not liquid that was originally present in the leaves.

The experiments reported here prove that absorption is an important factor in determining the amount of synthesis that occurs from infiltrated substrates and the centrifuging technique provides a useful tool in the further extension of infiltration studies.

Discussion

The vacuum infiltration technique provides the physiologist with a unique tool for the study of individual, and to a certain degree, isolated reactions in living plant tissue. It necessarily imposes, however, upon that tissue a set of highly artificial conditions. In effect the individual reaction under investigation is accentuated by increasing the concentration of the reactants to such a degree that the equilibrium is forced in the desired direction. In the foregoing sections certain data have been presented which were obtained by the infiltration technique and which throw considerable light on the sucrose synthesis mechanism of living wheat leaf tissue.

Although the in vivo synthesis of sucrose from single hexoses is not a photosynthetic process, it is closely associated with photosynthesis and fluctuates diurnally in a corresponding fashion. The reason for this close association is probably the concentration factor. The threshold concentration for initiating the transformation of hexoses to sucrose must be relatively low. The infiltration technique provides a means of artificially stimulating the reaction or shifting the equilibrium without depending upon photosynthesis.

The data presented in the section on substrate concentration indicate that under these artificial conditions the relation between substrate concentration and sucrose synthesis (i.e. velocity) does not have the appearance of a typical in vitro enzyme reaction. It shows an almost linear relationship right up to a concentration of glucose which is generally considered to be highly damaging

to plant tissue. The synthesis of sucrose goes considerably beyond the range found under natural conditions, showing that the potential capacity of the enzyme system is much greater than the rate of reaction associated with normal metabolism.

The substrate concentration relationship does not behave like a simple enzyme reaction because the final measured product is the resultant of a number of interacting factors. The prime factor is undoubtedly the enzymic conversion of glucose to sucrose but other factors are involved such as the absorption of substrate into the cells. It becomes evident that we are dealing with a complex physiological process.

The effect of temperature upon sucrose synthesis is likewise a complex relationship. In every case a low-temperature incubation resulted in a significant increase in sucrose content, but a low-temperature experience prior to infiltration produced a lowered sucrose content. The latter may be due to impairment of the synthesis mechanism but failure of the cells to absorb substrate or dislocation of the respiratory process are more probable factors.

The effect of relative humidity upon sucrose synthesis is not a simple process. At least two effects are apparent. In general the effect of dehydration upon respiration is that respiratory activity is depressed. The sugar equilibrium, however, is shifted in the direction of sucrose by dehydration. It appears therefore that the loss of water from the tissues has a different effect on the total respiratory mechanism than upon the sucrose synthesizing mechanism and the sum of these two is reflected in the varying amount of sucrose found under conditions of increasing desiccation.

A set of conventional results was obtained from the starvation experiments. They show that the sucrose-synthesizing mechanism passes through a series of changes until complete impairment occurs.

The penetration experiments have demonstrated that the cellular absorption of injected substrate is a continuous process over a period of several hours and may be a limiting factor in the synthesis of sucrose by the tissue. The centrifuging technique is a necessary and useful extension of the vacuum infiltration procedure for the study of tissue metabolism. By analyzing the centrifuged liquid it is possible to show how much substrate is retained by the leaf and this provides a measure of the efficiency of the synthetic mechanism.

Acknowledgment

The author wishes to acknowledge with gratitude the invaluable counsel of the late Professor G. H. Duff of the University of Toronto during the course of this research. He is also indebted to Dr. D. F. Forward for reviewing the manuscript.

References

1. Bidwell, R. G. S. 1951. Carbohydrate metabolism in plants. M.A. Thesis, Queen's Univ., Kingston, Ontario.
2. Blackman, F. F. 1904. Residual vitality. New Phytologist, **3**, 33–39.
3. Duff, G. H. and Forward, D. F. 1932. A survey of respiratory metabolism in the wheat plant. 1. The respiration of the isolated first seedling leaf. Proc. Roy. Soc. Can. Ser. 3, **26**, Appendix B: 107.

4. DUFF, G. H. and FORWARD, D. F. 1949. Physiological and biochemical studies in plant metabolism. 1. The respiration of the seedling wheat leaf in starvation and ontogeny. Can. J. Research, C, **27**, 125–145.
5. HANES, C. S. 1929. An application of the method of Hagedorn and Jensen to the determination of larger quantities of sugars. Biochem. J. **32**, 99–106.
6. HARTT, C. E. 1937. The synthesis of sucrose by excised blades of sugar cane. Hawaiian Planters' Record, **41**, 33–46.
7. HAWKEY, S. P. 1930. A study of injection in leaves. Ph.D. Thesis, Cambridge University, Cambridge, England.
8. KROTKOV, G. 1939. Carbohydrates and respiratory metabolism in the isolated starving leaf of wheat. Plant Physiol. **14**, 203–226.
9. KROTKOV, G. 1947. Vacuum infiltration as a method for determining enzymatic activity *in vivo*. Science, **105**, 318–319.
10. KROTKOV, G. and BENNETT, W. C. 1952. Synthesis and hydrolysis of sucrose by wheat leaves as determined by the vacuum infiltration method. Can. J. Botany, **30**, 28–39.
11. KURSANOV, A. 1936. The use of the vacuum infiltration method for determination of the synthetic and hydrolytic actions of invertase in living plant tissues. Biokyhimiya, **1**, 269–294.
12. MCCREADY, R. M. and HASSID, W. Z. 1941. Transformation of sugars in excised barley shoots. Plant Physiol. **16**, 599–610.
13. NELSON, J. M. and AUCHINCLOSS, R. 1933. The effect of glucose and fructose on the sucrose content of potato slices. J. Am. Chem. Soc. **55**, 3769–3772.
14. ROBERTS, D. W. A. 1951. Physiological and biochemical studies in plant metabolism. 3. The effects of starvation and mechanical stimulation on the respiratory metabolism of the first leaf of the wheat plant. Can. J. Botany, **29**, 383–402.
15. ROBERTS, D. W. A. 1951. Physiological and biochemical studies in plant metabolism. 4. Effect of illumination and darkness on the physiological heterogeneity of the first leaf of wheat. Can. J. Botany, **29**, 451–466.
16. VIRTANEN, A. I. and NORDLUND, N. 1934. Synthesis of sucrose in plant tissue. Biochem. J. **28**, 1729–1732.
17. WARD, G. M. 1952. The isolation and identification of sucrose from the seedling wheat leaf. Can. J. Botany, **31**, 75–80.
18. WARD, G. M. 1952. Physiological and biochemical studies in plant metabolism. VII. The invertase of the seedling wheat leaf. Can. J. Botany, **31**, 81–89.

EFFECT OF PHOTOPERIOD ON DROUGHT RESISTANCE OF WHITE SPRUCE SEEDLINGS[1]

O. VAARTAJA

Abstract

Seedlings of *Picea glauca* were grown for 2 months under three photoperiodic treatments. Short day treatment induced early terminal dormancy and resistance to severe drought treatment. Long day treatments, on the other hand, allowed prolonged growth of most seedlings and made them susceptible to drought.

Introduction

Since the experiments of Bogdanov (1) and Moshkov (3) it has been obvious that short days increase cold resistance in seedlings of many tree species. Therefore, it has been assumed that photoperiodic response in trees is an evolutionary adaptation to withstand the winter cold. It has also been suggested that the adaptation is not to a single factor, that is, low temperature, but rather to a complex of environmental factors, of which midsummer drought may sometimes be the principal one (4, 5).

Short days cause an early cessation of elongation of seedlings and make the tips of the stems dormant. Long days, on the other hand, prolong the growth cycle and, as a result, seedlings are taller and have a large transpiring surface. They would, therefore, be expected to be more susceptible to drought. On the other hand, long days also increase the root growth, which aids water uptake and tends to decrease the susceptibility. The balance of these two tendencies hardly can be calculated. Karschon (2) found that long days as compared with very short days (8 hour/day) slightly decreased the susceptibility of month-old seedlings of *Pinus sylvestris* L. and *P. montana* Mill.

In the following an experimental comparison is made between the drought resistance of well-established spruce seedlings grown under different photoperiods.

Methods

Stratified seeds of white spruce (*Picea glauca* (Moench) Voss) of Saskatchewan origin were germinated in sphagnum moss under continuous light. The young seedlings were planted in pots, 10 in each, in a mixture of moss, sterilized sand, and forest humus (5:4:1 ratio). The pots were kept in three air-conditioned greenhouse compartments that were fully exposed to sunlight during the day and in addition received artificial light during the night. Each compartment received the same amount of additional light from cool white fluorescent tubes (6000 ft-c hour per day), but with different timing as illustrated in Fig. 1. According to an earlier study (4) this arrangement provides photoperiodically different effects.

[1]Manuscript received March 16, 1960.
Contribution No. 624, Forest Biology Division, Research Branch, Canada Department of Agriculture, Ottawa, Canada; and No. 43, Canada Agriculture Research Station, Saskatoon, Saskatchewan.

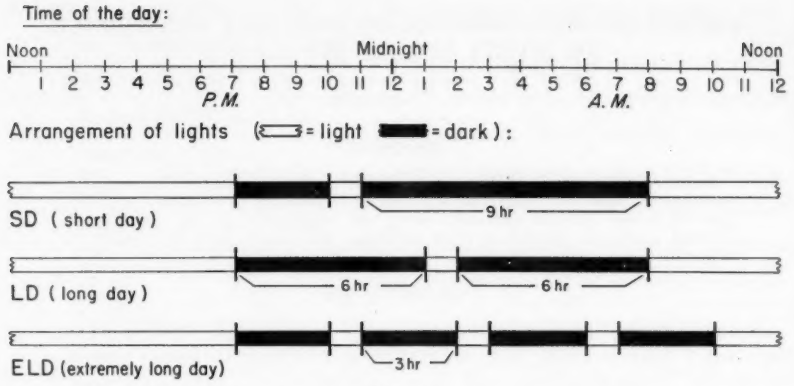

Time of the day:
Noon
Midnight
Noon
1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12
P.M.
A.M.
Arrangement of lights (= light = dark):
SD (short day)
9 hr
LD (long day)
6 hr
6 hr
ELD (extremely long day)
3 hr

FIG. 1

After 2 months of photoperiodic treatment, the pots were brought under continuous light and buried in dry vermiculite in sets of three, one pot from each treatment. The seedlings were deprived of water until severe drought symptoms appeared. Then the watering was started again, at first in one set and after a 4-day interval in the second set, and so on until all were watered. By this means the drought period was lengthened by 4 days in each successively watered set. The seedlings were thereafter kept well watered to test their ability to recover. This arrangement was made in the hope that, with some of the different drought periods, the differences between seedlings from different photoperiodic treatments would be large. The survivals were recorded a month later and the results analyzed by the Chi-square test.

Results

After the photoperiodic treatments all seedlings from the short day (SD) treatment showed a typical dormancy reaction and had developed terminal buds (Table I). Most seedlings from the long day treatments (LD and ELD) had no such reactions.

The effects of the desiccation treatment were clear cut (Table I). All seedlings grown under short days survived, but most seedlings grown under long days (LD and ELD) died. The differences between SD and the other treat-

TABLE I

Percentage of dormant white spruce seedlings and mortality due to desiccation treatment within each of three photoperiodic treatments[a]

Per cent of seedlings dormant			Per cent of seedlings dead		
ELD	LD	SD	ELD	LD	SD
3	3	100[b]	90	97	0[b]

[a]Treatments described in Fig. 1.
[b]The differences between seedlings grown under short days (SD) and either of the long days (ELD or LD) were significant at $P < 0.01$.

ments were significant (P much less than 0.01). Amongst the surviving seedlings were those few that became dormant under the long days. There was no difference between the results of the different drought periods. The seedlings grown under long days (LD and ELD) died quite soon and could not be revived even by the earliest waterings; the seedlings grown under short days, on the other hand, remained alive even past the longest drought period tested.

The experiment thus shows that short days may greatly increase the drought resistance of seedlings of white spruce. The resistance appears to be connected with the dormancy, but it is not yet known whether this is due to increased resistance of the tissue to desiccation or to smaller water loss, to both of these, or to other differences.

These results differed from those obtained by Karschon with pine seedlings (2). This may be due to the different species, or more likely to the different ages of the seedlings. The seedlings tested by Karschon were exposed to drought when very young, about a month old, when the small root system probably is the decisive factor in drought resistance. The seedlings of the present experiment were almost 3 months old when severely exposed. At that time the size of the root system probably is less important as a factor of drought resistance.

References

1. Bogdanov, P. 1931. Photoperiodism of trees. (In Russian.) Trudy Issledov Lesnomu Khoz. Lesnoi Premysh. **10**, 21–55.
2. Karschon, R. 1949. Untersuchungen über die physiologische Variabilität von Föhrenkeimlingen autochtoner Populationen. Mitt. schweiz. Anstalt forstl. Versuchswesen, **26**, 205–244.
3. Moshkov, B. S. 1935. Photoperiodismus und Frosthärte ausdauernder Gewächse. Planta, **23**, 744–803.
4. Vaartaja, O. 1959. Evidence of photoperiodic ecotypes in trees. Ecol. Monographs, **29**, 91–111.
5. Vaartaja, O. 1960. Ecotypic variation of photoperiodic response in trees especially in two Populus species. Forest Sci. In press.

INTERRELATIONSHIPS BETWEEN PLANT AGE, ROOT-ROT INFECTION, AND COLD HARDINESS IN WINTER WHEAT[1]

J. E. Andrews, J. S. Horricks, and D. W. A. Roberts

Abstract

The effect of plant age and root rot caused by *Bipolaris sorokiniana* and *Fusarium culmorum* on the cold hardiness of winter wheat and the effect of cold injury on root-rot infection were studied during 3 years at Lethbridge, Alberta. The oldest and youngest plants were less cold hardy than those of an intermediate age. Root-rot damage predisposed plants to cold injury and, in turn, cold injury predisposed plants to root-rot damage. Winter wheat inoculated with *B. sorokiniana* and *F. culmorum* and seeded at the earliest dates had recovered from infection before exposure to the freezing treatment and, apparently because of delayed development, was more cold hardy than uninoculated wheat seeded on the same date. Results from cold-chamber and field studies indicated that the severe root-rot damage often observed in nearly mature winter wheat seeded early the previous year is not a direct result of heavy infection at or near the time of seeding but is secondary to winter injury that often occurs in early-seeded winter wheat.

Introduction

Date of seeding is a very important factor in the economic production of winter wheat (1, 2, 5). Janssen (2), in Wisconsin, found that winter wheat seeded about September 18–22 survived better than that seeded on August 1 or on October 20. He attributed the lower survival of plants seeded on the latter date to damage from heaving. Their meager and shallow root system apparently rendered them more susceptible to this type of injury. He ascribed the low survival of wheat seeded on August 1 to some other but unknown cause. At Lethbridge, Alberta, a similar relationship has been observed (3). In an experiment involving weekly dates of seeding in 1955–56, winter wheat seeded September 6 and 13 survived with no apparent damage whereas that seeded 3 or more weeks earlier was winterkilled. Winter wheat that was seeded 4 weeks later and had just emerged from the soil at freeze-up was 80% winterkilled. Previous studies at the same location (5) also showed that common root-rot damage in mature plants is much more severe in winter wheat sown in August than in that sown later. Thus it is evident that early-seeded winter wheat is more subject to both root-rot damage and winter injury, but little is known concerning the influence of each of these factors on the other.

The experiments reported here were undertaken to study the effect of plant age and root-rot infection on cold injury and of cold injury on root-rot infection in winter wheat.

Materials and Methods

The experiments were carried out during the years 1956–1959. Two winter wheat varieties, Kharkov 22 M.C., which is very winter hardy, and Elgin,

[1]Manuscript received February 8, 1960.
Joint contribution from the Cereal Breeding and Botany and Plant Pathology Sections, Canada Agriculture Research Station, Lethbridge, Alberta.

which is much less winter hardy, were used in all experiments. To obtain hardened plants of different ages, the two varieties were planted at 2-week intervals in crocks or flats and allowed to harden outside. One series of each variety was inoculated with the common root-rot pathogens *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker and *Fusarium culmorum* (W. G. Sm.) Sacc. at or shortly after seeding. An uninoculated series served as controls. When plants from the earliest seeding were about 15 weeks old, plants of all ages were exposed simultaneously to freezing in controlled temperature chambers. Since hardening conditions and, consequently, the degree of hardening attained varied from year to year, pilot freezing tests of field-hardened wheat were carried out each year at different temperatures and durations. The selection of an appropriate freezing treatment was based on the results of these pilot tests. In other respects the experiments differed somewhat from year to year and the procedure in each is described below.

The 1956 experiments were carried out in flats 18×24×6 in. In each flat one variety was seeded on five dates at 2-week intervals beginning August 1. At each seeding date 20 seeds were sown in a single row 18 in. long in random position in each flat. Each of the two varieties was studied in a separate experiment of split-plot design involving inoculated and uninoculated treatments and the five seeding dates. There were 15 replications. To prepare inoculum, *B. sorokiniana* was grown for 2 weeks at 25° C in a medium of 10% corn meal in a 3:1 soil–sand mixture. Twenty-five grams of inoculum were added to each row of week-old seedlings at seed level. The 15 replications of each experiment were divided into three groups of five and each was exposed separately to freezing. The freezing treatment for Elgin was exposure to −20° C for 22 hours and for Kharkov 22 M.C. −25° C for 24 hours.

The 1957 experiments were carried out in 1-gal crocks with one date of seeding and one inoculation treatment per crock. Twelve seeds were planted per crock, and the plants, except those seeded on the last two dates, were thinned at the two-leaf stage to six per crock to avoid overcrowding. There were five seeding dates at 2-week intervals beginning August 8. At each date there were paired crocks of inoculated and uninoculated treatments. To prepare inoculum, *B. sorokiniana* and *F. culmorum* were grown separately in potato dextrose broth for 2 weeks on a rotary shaker at 25° C. The cultures of both fungi were macerated in a Waring blender, mixed to form a composite inoculum, and diluted 1:1 with water. One hundred milliliters were added to each crock at seed level at the time of seeding. Seeds were then covered with soil to a uniform depth. Each of the two wheat varieties was grown in a separate series with 10 replications for freezing studies and four for root-rot studies prior to the time of freezing. The freezing treatments for the first five replications were begun on November 25, Elgin at −15° C for 40 hours and Kharkov 22 M.C. at −20° C for 40 hours, and for the second five replications on December 9, Elgin at −15° C for 72 hours and Kharkov 22 M.C. at −20° C for 48 hours. The second five replications were exposed for a longer period because the first freezing treatment did not appear to have been sufficiently severe.

The 1958 experiments also were carried out in crocks, with six seeding dates at 2-week intervals beginning July 29. The methods of seeding and inoculation were similar to those used in 1957 except that 100 ml of undiluted inoculum were used per crock. Ten replications were used for the freezing tests, four for root-rot ratings prior to the time of freezing, and four as unfrozen checks in the greenhouse. The freezing treaments for five of the replications were begun on November 6, Elgin at −15° C for 30 hours and Kharkov 22 M.C. at −20° C for 48 hours. For the other five replications the freezing treatments were begun on November 26, Elgin at −15° C and Kharkov 22 M.C. at −20° C, both for 48 hours.

Root-rot ratings in 1957 and 1958 were made both before and after freezing. Seedlings were carefully dug up, washed, and rated for root-rot on a scale of 0 (undamaged) to 10 (killed). It was not possible to expose to freezing the same plants that had been examined for root rot. Four replications were used for root-rot ratings prior to freezing and it was assumed that the means of these ratings were reliable estimates of root-rot infection in plants exposed to freezing. Surviving plants were rated for root rot 4 weeks after freezing in 1957 and 6 weeks after freezing in 1958. In 1958, check plants grown under similar conditions but not exposed to freezing temperatures were rated at the same time to assess root-rot development in plants not injured by cold.

Survival percentages were based on plant counts in 1956 and on tiller counts in the remaining years. Tillers were counted immediately prior to exposure to freezing and surviving tillers 3 to 5 weeks after exposure to freezing. In the 1958 tests the number of tillers per plant in four replications of unfrozen checks also was counted to determine the normal increase in the number of tillers during the period between exposure to freezing and tiller counting. On this basis the expected number of tillers, if there had been no damage from freezing, was determined and used in calculating survival percentages.

Results

Cold-Chamber Studies

The use of flats for growing rows of plants seeded on the five different dates in the 1956 experiments proved unsatisfactory in some respects. Later-seeded plants did not compete well when seeded between the earliest-seeded rows, and the large flats presented physical difficulties in the field and in the freezing chambers.

The survival percentages for the various treatments are given in Table I. Plants seeded on the last date were in the three-leaf stage and those seeded on the first date had 10 or more tillers. The analysis of variance indicated a significant difference ($P<0.01$) between dates of seeding and a significant interaction ($P<0.01$) between dates and inoculations for both varieties. The interaction between dates of seeding and inoculation with *B. sorokiniana* was the most important feature of these results. Survival of inoculated plants of both varieties was lower than that of the uninoculated ones at the later dates of seeding and higher (Elgin) or about the same (Kharkov 22 M.C.) at

the earlier dates of seeding. These results were contrary to expectation based on the results of previous field tests in which plants from the earliest dates of seeding were most severely infected with root rot and the theory that this might predispose them to winterkilling. The explanation became apparent in later experiments. For uninoculated Elgin, survival of plants seeded at the earliest date was lowest and increased progressively for plants seeded later. For Kharkov 22 M.C., although there was a significant difference in survival between plants from different dates of seeding, plants from all dates of seeding suffered severe damage and it probably is unwise to interpret the effect of stage of development on cold hardiness from this experiment.

TABLE I

Survival of two winter wheat varieties at various stages of development, inoculated and uninoculated with *B. sorokiniana* and exposed to freezing temperatures (Elgin at −20° C for 22 hours and Kharkov 22 M.C. at −25° C for 24 hours)

	Date of seeding					
	Aug. 1	Aug. 15	Aug. 30	Sept. 12	Sept. 26	
Variety and treatment	Survival percentage					S.E.
Elgin						
Inoculated	26.0	36.6	43.2	30.3	44.8	4.3
Uninoculated	20.6	27.8	36.6	36.9	47.5	
Kharkov 22 M.C.						
Inoculated	13.4	24.0	10.6	4.0	23.3	3.3
Uninoculated	15.7	26.3	18.7	18.4	33.7	

Root-rot percentages from the 1957 experiments are given graphically in Fig. 1 (top). Since most of the inoculated Elgin plants from the September 19 seeding died soon after inoculation no survival or root-rot data were obtained for this treatment. The percentage of root rot prior to freezing in Kharkov 22 M.C. was higher ($P<0.05$) in inoculated than in uninoculated plants seeded September 19 and October 3. In Elgin it was higher ($P<0.01$) in inoculated than in uninoculated plants seeded August 22 and September 5.

The survival percentages from the 1957 experiments are shown graphically in Fig. 1 and the "P" values from the analysis of variance are given below.

	Variety and date frozen			
	Kharkov 22 M.C.		Elgin	
Source of variation	Nov. 25	Dec. 9	Nov. 25	Dec. 9
Dates (A)	$<.01$	$<.05$	$<.05$	$<.05$
Inoc. vs. uninoc. (B)	$>.05$	$<.01$	$<.01$	$<.05$
Interaction A × B	$>.05$	$<.01$	$<.05$	$<.05$

The survival of uninoculated plants of both varieties at the two freezing times was lower for the earliest and latest seeding dates than that for the intermediate dates (Fig. 1). There was no difference in survival between inoculated

and uninoculated Kharkov 22 M.C. exposed to freezing November 25. Survival of inoculated plants was significantly lower than that of uninoculated plants of Kharkov 22 M.C. exposed to freezing on December 9 and of Elgin exposed to freezing on November 25 and December 9 (Fig. 1), and in these cases there

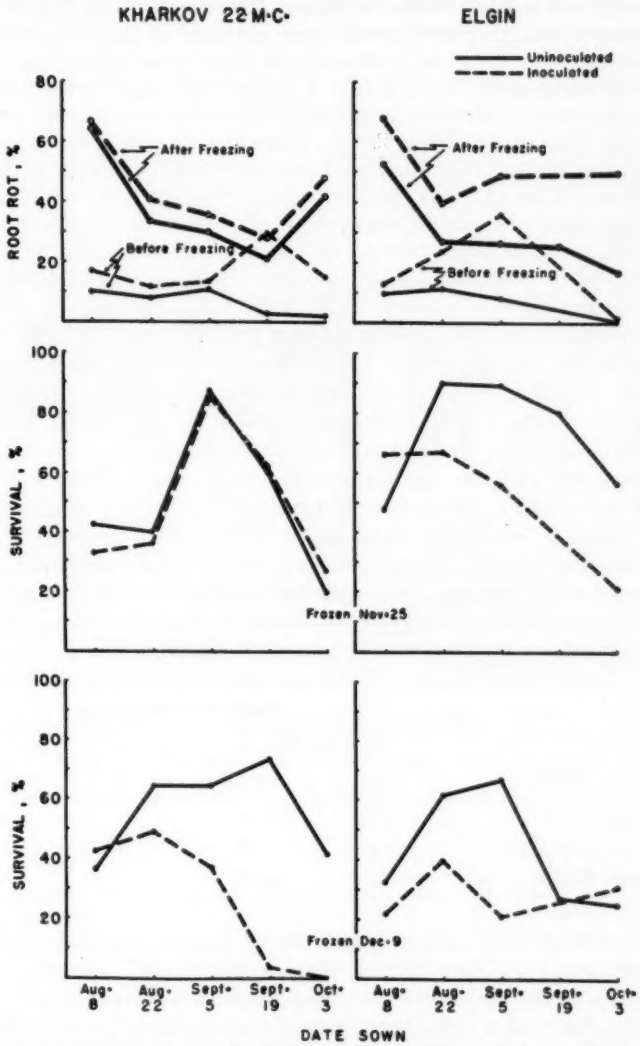


FIG. 1. Percentage survival of two winter wheat varieties seeded on different dates, inoculated and uninoculated with root-rotting organisms, and exposed to freezing temperatures; percentage root rot immediately before exposure to freezing temperatures and 4 weeks after the November 25 exposure.

was a significant interaction between dates and inoculations. Differences in survival between inoculated and uninoculated plants corresponded closely to differences in root-rot infection prior to freezing (Fig. 1). Survival of inoculated plants from the earliest seeding date of Kharkov 22 M.C. frozen December 9 and of Elgin frozen November 25 was higher than that of uninoculated plants (Fig. 1). Similar results were obtained in the 1956 experiment.

Plants surviving from the November 25 freezing were rated for root rot about four weeks later. The data (Fig. 1, top) showed that there was a greater increase in root-rot infection in plants in which freezing damage was most severe (i.e., earliest and latest dates). There was no significant difference in root-rot infection between inoculated and uninoculated plants of Kharkov 22 M.C., which also had not been damaged differentially by freezing. There was a significant difference in root-rot infection between inoculated and uninoculated plants of Elgin ($P<0.05$), which previously had been damaged differentially by freezing. This shows that cold injury predisposes plants to root-rot injury.

The results of the 1958 experiments were similar in most respects to those obtained in the previous years. The stage of development of typical plants seeded at each date and the relative root-rot damage immediately prior to freezing are shown in Fig. 2. Root-rot percentages are shown graphically in Fig. 3 (top). Plants seeded and inoculated on the first two dates were heavily infected with root rot initially but later recovered from the infection and, when exposed to freezing, were apparently as healthy as uninoculated plants. Infection was significantly higher ($P<0.01$) in inoculated than in uninoculated plants of both varieties seeded on the last four dates (Fig. 3).

The survival percentages after exposure to freezing are shown graphically in Fig. 3. The "P" values obtained from the analysis of variance are given below.

	Variety and date frozen			
	Kharkov 22 M.C.		Elgin	
Source of variation	Nov. 6	Nov. 26	Nov. 6	Nov. 26
Dates (A)	$<.01$	$<.01$	$<.01$	$<.01$
Inoc. vs. uninoc. (B)	$>.05$	$>.05$	$>.05$	$>.05$
Interaction A × B	$<.01$	$<.01$	$<.01$	$<.01$

Survival of uninoculated plants (Fig. 3) seeded on the earlier dates and on the later dates was lower than that of plants seeded on the intermediate dates. There was a significant interaction between inoculations and dates of seeding in all tests. Survival of inoculated plants seeded on the earlier dates of seeding was higher than that of the corresponding uninoculated plants, and, conversely, survival of inoculated plants seeded on the later dates of seeding was lower than that of the corresponding uninoculated plants. The mean

PLATE I

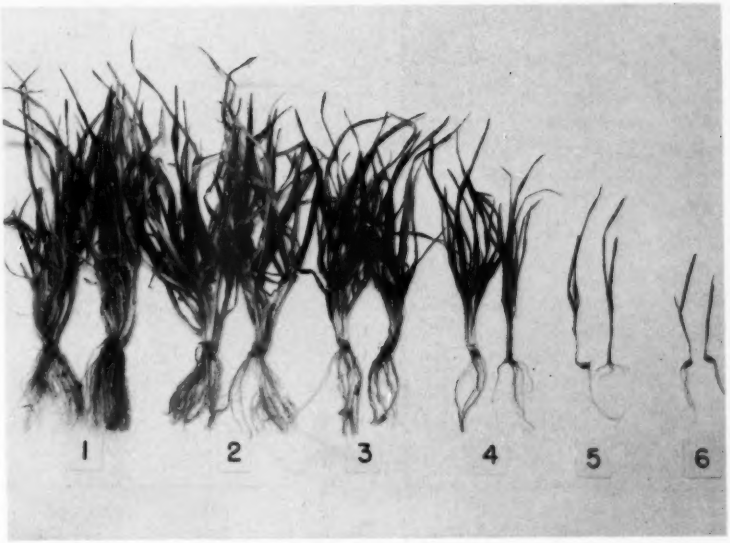


FIG. 2. Relative stages of development and root-rot damage of winter wheat seeded on six dates beginning July 29 and at intervals of 2 weeks thereafter; left plant of each pair was uninoculated, right plant inoculated.

survival of inoculated plants seeded on all dates was not significantly different from that of uninoculated plants, as might be expected because of the interaction. However, survival of inoculated plants was significantly lower ($P<0.05$) than that of uninoculated plants in each of the following cases.

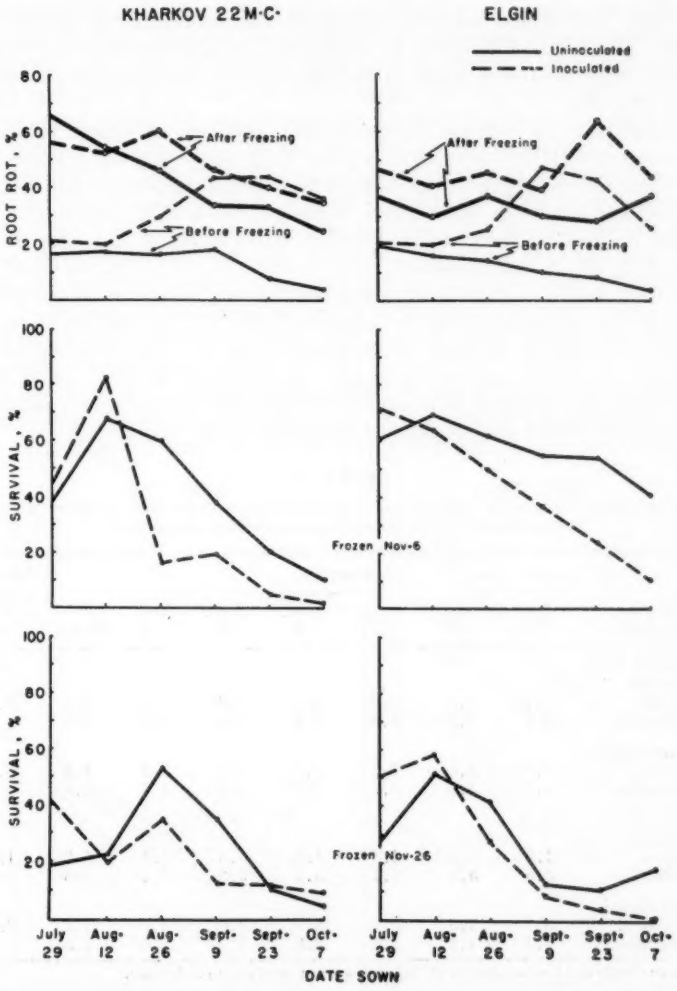


FIG. 3. Percentage survival of two winter wheat varieties seeded on different dates, inoculated and uninoculated with root-rotting organisms, and exposed to freezing temperatures; percentage root rot immediately before exposure to freezing temperatures and 6 weeks after the November 6 exposure.

Variety	Date seeded	Date frozen
Elgin	Aug. 26, Sept. 9 and 23, Oct. 7	Nov. 6
Kharkov 22 M.C.	Aug. 26, Sept. 9 and 23, Oct. 7	Nov. 6
Elgin	Aug. 26, Sept. 9 and 23, Oct. 7	Nov. 26
Kharkov 22 M.C.	Aug. 26, Sept. 9	Nov. 26

Differences in survival between inoculated and uninoculated plants corresponded closely to differences in their root-rot infection prior to freezing, survival being relatively lower in treatments with more severe root-rot infection (Fig. 3).

A comparison of root-rot infection after exposure of plants to freezing temperatures with that immediately prior to exposure is given graphically in Fig. 3 (top) and with checks not exposed to freezing temperatures in Table II. Root-rot infection was much more severe 6 weeks after plants had been exposed to freezing than before freezing, particularly in the case of older plants (Fig. 3). A comparison of root-rot infection in plants that had been frozen with that in unfrozen controls gives a better indication of the effect of cold injury on subsequent root-rot damage (Table II). The analysis of variance showed a significant increase ($P<0.01$) in root rot after freezing in both varieties, inoculated and uninoculated. In Kharkov 22 M.C. particularly, this increase was greatest in the earlier-seeded plants, which were larger and of which there was a greater number partly damaged by freezing.

TABLE II

Relative root-rot damage in inoculated and uninoculated winter wheat seeded on different dates and exposed or not exposed to freezing temperatures

Variety and treatment	Date seeded: July 29	Aug. 12	Aug. 26	Sept. 9	Sept. 23	Oct. 7	Mean	Min. sig. range at one date, $P = .05$
Kharkov 22 M.C.								
Inoculated								
Frozen	5.7*	5.3	6.5	4.7	4.0	3.5	5.5	1.5
Unfrozen	4.3	3.9	4.6	3.8	3.8	3.8	4.2	
Uninoculated								
Frozen	7.0	5.8	4.6	3.5	3.4	2.5	4.5	0.8
Unfrozen	3.4	3.3	3.3	3.3	2.3	1.7	2.9	
Elgin								
Inoculated								
Frozen	4.6	4.2	4.8	4.1	6.5	4.4	4.8	1.6
Unfrozen	3.6	3.4	4.7	3.7	3.8	3.1	3.8	
Uninoculated								
Frozen	3.8	3.0	3.7	2.9	2.9	3.2	3.2	0.7
Unfrozen	3.2	3.1	3.4	2.8	2.3	1.9	2.8	

*Rated on a scale of 0 (undamaged) to 10 (killed) 6 weeks after exposure to freezing.

Field Studies

Natural root-rot development in Kharkov 22 M.C. wheat seeded on different dates on irrigated land also was studied in the same years in which the cold-chamber studies were carried out. In the fall of 1956 Kharkov 22 M.C. was

seeded at weekly intervals in a four-replicate test beginning August 1 and ending October 17. There was 50% winterkilling of plants seeded on the first date and 12.5% on the second. There was also evidence of partial cold damage to surviving plants that had been seeded prior to the first week of September. Plants from each seeding date were dug up soon after growth began in the spring and examined for root-rot damage. There was no apparent difference in root-rot damage between plants seeded on different dates. However, by heading time in June the wheat seeded prior to the first week in September showed symptoms of severe root-rot damage, with the most damage in the earliest-seeded wheat. This suggested that the cold injury in the early-seeded wheat predisposed the plants to root-rot infection in the spring.

In the 1957–58 and 1958–59 crop years natural root-rot infection again was studied in dates-of-seeding tests of Kharkov 22 M.C. winter wheat on irrigated land. Plants were examined for root rot in the late fall or early spring and on three other dates before maturity. The data obtained are shown graphically in Fig. 4. With the exception of the rating on July 15, 1959, the root-rot percentages were based on root-rot ratings of 10 plants taken at random from each of four replications. The July 15, 1958, rating was based on 20 plants per replication.

The winter of 1957–58 was mild and there was little or no winterkilling, but there was some evidence of cold injury in the form of leaf necrosis to plants in the earliest-seeded plots. In the 1958–59 test about 15% of the plants were

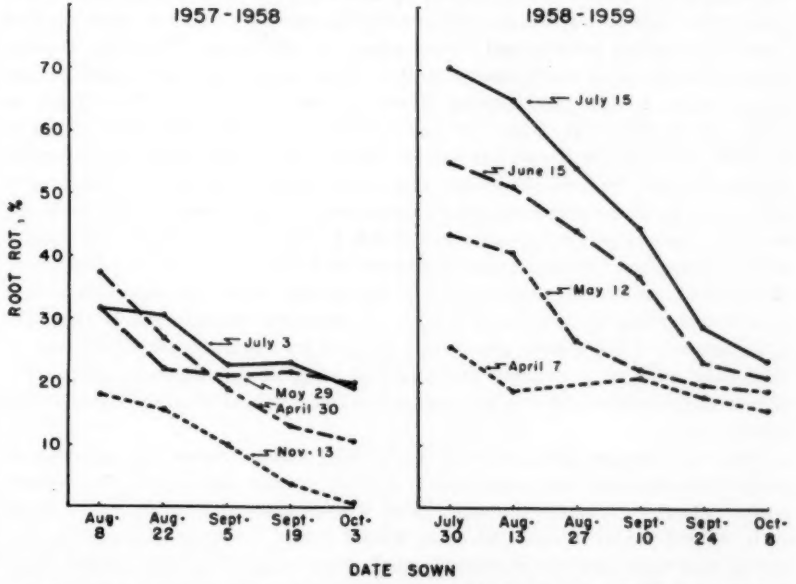


FIG. 4. Percentage root-rot damage at various sampling times in Kharkov 22 M.C. seeded on different dates on irrigated land in the 1957–58 and 1958–59 crop years.

winterkilled in the earliest-seeded plots. There was partial damage to plants seeded in August and no apparent damage to those seeded later. In both years there was a significant difference ($P<0.01$) in root-rot infection between seeding dates and between sampling dates (Fig. 4). In the 1957–58 years, when there was little winter damage, there was a higher percentage of root rot in the spring and early summer than in the late fall but the increase that occurred during the summer was about the same as that in plants seeded on all dates. Plants seeded at the earlier dates had the highest percentage of root rot at all sampling times. In the 1958–59 year, when plants seeded at the earlier dates showed some winter injury, root-rot infection increased more rapidly during the spring and early summer in plants seeded on the earlier than on the later dates (Fig. 4). A comparison of the data from the 2 years indicates that root rot becomes most severe in winter wheat that has been partly damaged by cold.

Discussion

The data from the cold-chamber studies showed that older (seeded early) and younger (seeded late) winter wheat plants are less cold hardy than those of an intermediate age. These results are similar to those obtained in the field (1, 2, 3). No attempt was made to compare results on actual calendar dates either between experiments or between cold-chamber and field results because hardening conditions outside varied greatly from year to year and the date on which plants were exposed to freezing also varied. Cold injury was greatest in plants that were most severely damaged by root rot, showing that root-rot infection predisposed winter wheat to cold injury. However, the low winter hardiness of early-seeded winter wheat cannot be attributed to any great extent to the predisposing effects of root-rot infection but rather to stage of development. Root rot inoculation studies showed that, although initially infected, early-seeded winter wheat recovered from the infection during the fall. Sallans (4) found that spring wheat may recover from early infections by *B. sorokiniana* and *F. culmorum*. In the present study, in 9 out of 10 analyses of percentage survival (Table I, Figs. 1 and 3) there was a significant interaction between dates of planting and inoculation vs. uninoculation. Survival of inoculated plants seeded at the earliest dates was higher than that of corresponding uninoculated plants. A probable explanation is that the development of inoculated plants was delayed by initial root-rot infection so that they were at a younger physiological stage of development than their uninoculated counterparts when exposed to freezing and, therefore, more cold hardy.

Root-rot damage after exposure to freezing temperatures was greatest in plants that had the most cold injury, indicating that cold injury predisposes winter wheat to root-rot damage. Field studies showed that, in years when early-seeded winter wheat had some winter injury, root-rot infection in the spring and early summer increased much more rapidly in early-seeded than in late-seeded winter wheat. When there was no apparent winter injury the increase in root-rot damage during the spring and early summer was about

the same in wheat seeded on all dates. Thus it appears that the severe root-rot damage that is often observed near harvest time in winter wheat that had been seeded early does not result directly from its being seeded at a time when the environment is conducive to infection. It occurs more likely because the early-seeded wheat is less cold hardy and, when partially damaged by cold, it is more susceptible to root rot. Under these conditions severe root-rot damage in spring and summer would be secondary to the cold injury.

Acknowledgments

The authors wish to thank Drs. M. W. Cormack and E. J. Hawn for assistance with root-rot examinations on the 1956–57 field test.

References

1. ANDREWS, J. E., PITTMAN, U. J., HORRICKS, J. S., HOLMES, N. D., ANDERSON, D. T., and SMITH, A. D. 1959. Winter wheat production in Western Canada. Can. Dept. Agr. Publ. 1056.
2. JANSSEN, G. 1929. Effect of date of seeding of winter wheat on plant development and its relationship to winterhardiness. J. Am. Soc. Agron. **21**, 444–466.
3. PITTMAN, U. J. and ANDREWS, J. E. Unpublished.
4. SALLANS, B. J. 1959. Recovery in wheat from early infections by *Helminthosporium sativum* and *Fusarium culmorum*. Can. J. Plant Sci. **39**, 187–193.
5. SLYKHUIS, J. T., ANDREWS, J. E., and PITTMAN, U. J. 1957. Relation of date of seeding winter wheat in southern Alberta to losses from wheat streak mosaic, root rot, and rust. Can. J. Plant Sci. **37**, 113–127.

GROWTH AND METABOLISM OF ASPERGILLUS NIDULANS EIDAM ON DIFFERENT NITROGEN SOURCES IN SYNTHETIC MEDIA CONDUCIVE TO FAT FORMATION[1]

KAISER NAGUIB AND KAMEL SADDIK

Abstract

Aspergillus nidulans has been grown in surface culture on high-sugar media favorable for fat formation and containing, as nitrogen source, sodium nitrate, ammonium chloride, or asparagine. Growth, sugar and nitrogen uptake, and syntheses of carbohydrates, proteins, and fat were all followed over an incubation period of 20 days. In the early stages, growth was influenced by the nature of the nitrogen source, being highest on asparagine and lowest on ammonium chloride, with the same sugar uptake. Later, dry weight increase proceeded at a high rate on asparagine, at a moderate rate on sodium nitrate, and it almost stopped on ammonium chloride, where the pH dropped to a very low value. At this stage, increase in dry weight followed sugar absorption, and was due to accretion of non-nitrogenous compounds.

Asparagine media were by far superior to nitrate or ammonium media for fat formation. Protein and carbohydrate contents were higher in nitrate- than in asparagine- or ammonium-fed mycelial felts. All synthetic processes almost stopped in ammonium cultures after the early growth phase. It seems that the attitude of the fungus towards ammonium nitrogen could not be fully manifested due to restricted growth, and therefore it is suggested that no definite conclusions with regard to ammonium utilization by *Aspergillus nidulans* can be drawn unless the pH of the medium is controlled and growth on ammonium nitrogen made possible.

Introduction

It is now already established that a high C:N ratio is highly conducive to fat formation by several microorganisms (Prill and co-workers (21), Heide (10), Bichkovskaya (2), Kleinzeller (13), and Naguib and Walker (19)). The effect of the nature of the carbon source on growth and fat formation has also been investigated by many workers such as Lockwood and co-workers (16) with *Penicillium javanicum*, Reichel and Reinmuth (22) with *Endomyces vernalis*, Kaibara (12) with *Penicillium javanicum*, Naguib (17) with *Aspergillus nidulans*, and with *Penicillium lilacinum* (18).

As regards the effect of the nitrogen source on growth and development of fungi, different conclusions have been arrived at depending on several factors one of which is the organism under investigation. Robbins (23) and Steinberg (27, 28) have reviewed a good deal of work on this subject. It was found that fungi differ in their behavior towards the utilization of nitrate vs. ammonium nitrogen as well as their preference of either organic or inorganic nitrogen sources.

It has also been pointed out in several instances that the source of nitrogen may exert an influence on the total nitrogen content of the mycelium. Thus, Tamiya (32) has shown that ammonium salts lead to higher nitrogen content than nitrates. Subramanian and Pai (30), on the other hand, obtained higher

[1]Manuscript received March 22, 1960.
Contribution from the Botany Department, Faculty of Science, Cairo University, Egypt, U.A.R.

total nitrogen content in mycelium of *Fusarium vasinfectum* fed with sodium nitrate than when fed with ammonium sulphate. In higher plant tissues, Street and co-workers (29) reported that the protein content of potato sprouts supplied with nitrate was higher than when supplied with ammonium salts.

In relation to fat synthesis, Garrido and Walker (8) have shown that *Aspergillus nidulans*, *Penicillium javanicum*, and *Penicillium spinulosum* behave differently towards different nitrogen sources. Philip and Walker (20), using *Penicillium lilacinum*, arrived to a similar conclusion.

It was thought in the present inquiry to study in some detail the effect of three different nitrogen sources, namely, sodium nitrate, ammonium chloride, and asparagine on growth and metabolism of the fat-forming fungus *Aspergillus nidulans* by tracing the synthesis of proteins and fat as well as the accumulation of carbohydrates when grown in surface culture during a reasonable period of incubation.

Materials and Methods

The Organism

The fungus used in the present work was *Aspergillus nidulans* Eidam, obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland. It was maintained on slant cultures of Dox's agar medium and was subcultured every 2 weeks.

Composition of Media and Cultural Conditions

The fungus was grown as surface cultures on a basal liquid medium of the following composition (g/100 ml): Glucose, 17; sodium dihydrogen phosphate, 0.73; potassium sulphate, 0.022; magnesium sulphate, 0.500; zinc sulphate, 0.005; and ferric chloride, 0.016. This basal medium together with ammonium nitrate as nitrogen source was proved to be favorable for fat formation by *Aspergillus nidulans* (Garrido and Walker(8)). To complete the medium, the nitrogen source was added in the form of sodium nitrate (6.07 g/l.), ammonium chloride (3.82 g/l.), or asparagine (4.71 g/l.) on equal nitrogen bases. Three media were thus prepared, adjusted to pH 3.8, and made up to volume with distilled water. They were then distributed into 150-ml conical flasks receiving each 25 ml of medium. The three sets of flasks were plugged and autoclaved at 10 lb for 15 minutes. After cooling, they were inoculated with a spore suspension made up from 2-week-old slant cultures on Dox's agar medium. The culture flasks were incubated at 25° C. After 4, 6, 8, 10, 13, 16, and 20 days, quadruplicate sets of culture flasks were withdrawn. The total nitrogen and residual sugar were estimated in the culture media, while the mycelial felts were dried and weighed, and were further analyzed for their total nitrogen, protein, fat, and total carbohydrates.

Methods of Analysis

Estimations of residual sugar and total nitrogen in the culture media, and of carbohydrates, proteins, and fat in the mycelial felts, were conducted by procedures referred to in a previous communication (Naguib (17)).

Results

Sugar Uptake

Absorption of glucose from all the culture media, as can be deduced from Fig. 1, proceeded at the same rate for the first 4 days of incubation. Later, slight absorption was detected in the ammonium chloride cultures, while in cultures provided with asparagine, the sugar content was almost completely exhausted in 13 days. In sodium nitrate cultures, complete absorption took place by the 20 days of incubation.

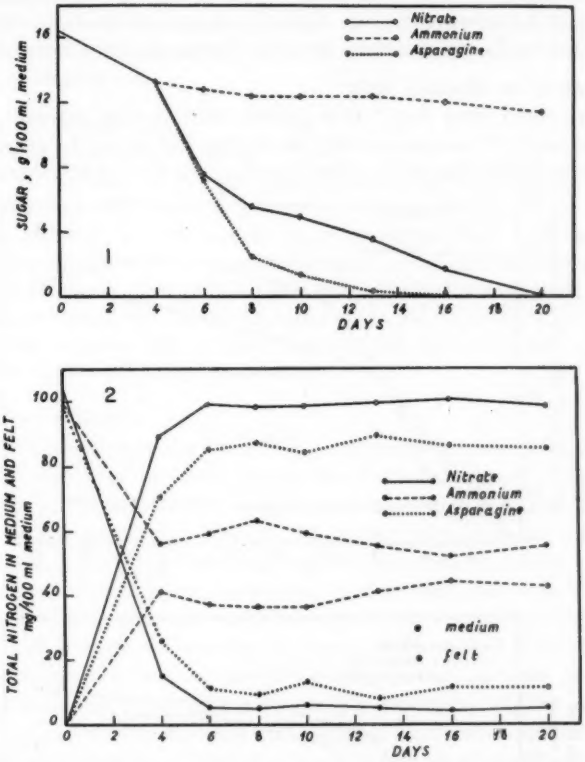


FIG. 1. The sugar concentration of the culture media (expressed as grams glucose) during the incubation period.
FIG. 2. The total nitrogen content of the different media and mycelial felts during the incubation period.

Nitrogen Uptake and Accumulation in the Mycelial Felts

Absorption of nitrogen (Fig. 2) proceeded at a relatively low rate in the ammonium chloride cultures and completely stopped by the fourth day, although an ample amount of nitrogen was still remaining in the external media. In the nitrate as well as in the asparagine media, absorption started

very actively and continued until the sixth day when only a small fraction of the nitrogen supply was left. The rate of nitrogen uptake from the nitrate media was markedly higher than from the asparagine media.

The drifts in the nitrogen content of the culture media were reflected in the nitrogen contents of the mycelial felts as can also be noted from Fig. 2. The rate of building up of nitrogenous compounds from asparagine or nitrate media was higher than from ammonium chloride media. The figure also indicates that, in the ammonium chloride cultures, there was a release of nitrogen from the mycelial felts into the external media during the incubation period from the fourth to the eighth day. A simultaneous increase in the nitrogen content of these media could also be detected during the same interval.

Dry Weights of the Mycelial Felts

It can be noted from Fig. 3 that growth started most actively on nitrate media and least on ammonium chloride media. But later, the growth rate on asparagine exceeded that on the nitrate media to such an extent that the highest

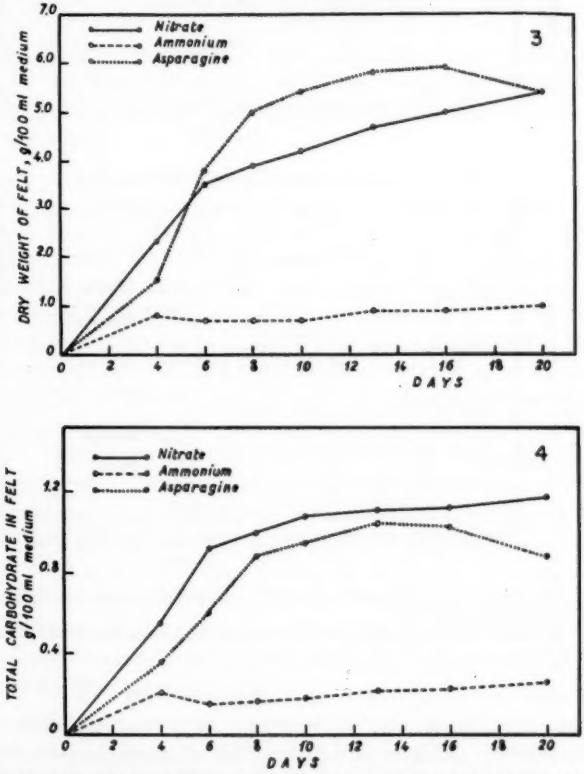


FIG. 3. The dry weights of the mycelial felts during the incubation period.
FIG. 4. The total carbohydrate content of the mycelial felts during the incubation period (expressed as grams glucose).

yield of mycelium was obtained on asparagine by the 16th day. After this interval, growth declined on asparagine but continued on nitrate where it reached its maximum by the end of the experimental period. Concurrent with this was the trend in the sugar content of the culture media. Sugar completely disappeared on the 16th day from the asparagine media and by the 20th day from the nitrate media.

On the other hand, growth on ammonium chloride proceeded only for 4 days after which it almost completely stopped. It is interesting here to remember that analysis of the culture media has shown that after the first 4 days of incubation, no more nitrogen was taken up from the ammonium chloride media; only very small amounts of sugar were absorbed probably to maintain a low rate of respiration.

Analysis of the Mycelial Felts

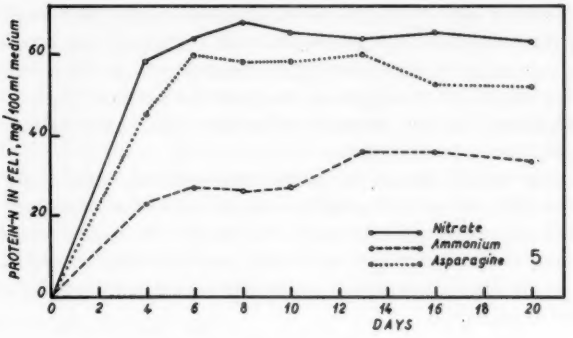
1. The Total Carbohydrate Content

Figure 4 shows that bigger amounts of carbohydrates accumulated in the mycelial felts fed with sodium nitrate as compared with those fed with ammonium chloride or asparagine. In cultures supplied with ammonium chloride, very small amounts of carbohydrates accumulated during the first 4 days of incubation after which the total carbohydrate content remained almost constant until the end of the experiment. As a matter of fact, the dry weight of the mycelial felt in these cultures did not show any appreciable change after the fourth day of incubation.

2. The Protein Content

Protein synthesis, as indicated by Fig. 5, proceeded actively right from the beginning. It continued until the eighth day in the nitrate cultures, and until the sixth day in the ammonium chloride and asparagine cultures. The rate of synthesis was highest from nitrate and lowest from ammonium chloride. On asparagine, protein synthesis was slightly less than on nitrate. Moreover, it can be noted that the protein content of the nitrate-fed mycelial felts remained later almost constant until the end of the experimental period, while on asparagine, it remained constant for some time and then proteolysis set in. It may be recalled here that the dry weight of the mycelial mats of the nitrate cultures continued to increase until the expiry of the experiment, while on asparagine the mycelial mats started autolysis some days earlier. With ammonium chloride a further increase in protein content occurred later, followed by a slight decline.

When the protein content was calculated on basis of percentage dry weight of the mycelial felt (Table I), it was noted that ammonium chloride as a nitrogen source gave rise to remarkably higher protein synthesis than nitrate or asparagine throughout the experimental period. Since there was lower increase in dry weight of the mycelial felts on ammonium chloride, this high percentage may be attributed to the very low accumulation of non-nitrogenous compounds in the mycelium.



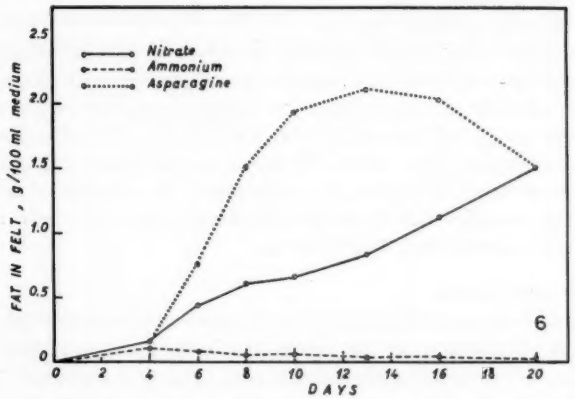


FIG. 5. The protein nitrogen content of the mycelial felts during the incubation period.
FIG. 6. The fat content of the mycelial felts during the incubation period.

3. The Fat Content

As can be noted from Fig. 6, the fat content of the mycelial felts grown on different nitrogen sources remained low during the first 4 days of incubation. No fat synthesis could further proceed in the ammonium chloride cultures and only negligible amounts of fat could be detected. But in both nitrate and asparagine cultures active fat synthesis started by the fourth day and proceeded at a remarkably higher rate in the asparagine than in the nitrate cultures. Fat accumulation reached its maximum on asparagine by the 13th day, and then it declined. At this maximum, the mycelial felt contained about 37% fat (Table I). In the nitrate cultures, where fat synthesis went on at a relatively low rate, the maximum fat content was reached by the end of the experimental period. At this time of harvest, the nitrate-fed mycelial felts had the same dry weight and the same fat content as the asparagine-fed ones. The percentage fat yield was at that time in both cases about 28%. This result shows clearly, as has been pointed out by Lilly and Barnett (14) and

Cockrane (5), that experiments in fungal physiology planned with a single harvest at the expiry of the experimental period may give misleading conclusions.

TABLE I

Change in percentage (dry weight basis) protein and fat in mycelial felts of *Aspergillus nidulans* when grown on sodium nitrate, ammonium chloride, or asparagine during the incubation period

Medium	Days of incubation						
	4	6	8	10	13	16	20
				Protein			
Nitrate	16.0	14.2	10.9	9.8	8.2	8.1	7.2
Ammonium	19.9	25.4	24.6	24.0	25.9	25.5	21.0
Asparagine	19.6	9.7	7.2	6.0	6.4	5.3	5.9
				Fat			
Nitrate	6.4	12.8	14.6	17.2	19.6	23.6	28.0
Ammonium	6.7	3.9	4.5	5.2	5.1	5.4	4.5
Asparagine	9.6	20.4	30.4	35·6	37.2	35.2	28.4

It is evident, under the present experimental conditions, that asparagine was highly superior to nitrate for fat formation by *Aspergillus nidulans*. Garrido and Walker (8) using the same fungus did not get much difference in fat yield when feeding with sodium nitrate or urea. The same authors also found that, while *Penicillium javanicum* yields heavier mycelium and more fat with sodium nitrate than with ammonium chloride, *Penicillium spinulosum* grows better and yields more fat on ammonium chloride than on sodium nitrate. However, no conclusion regarding the inability of the present fungus to build up fat in the presence of ammonium chloride as a nitrogen source can be drawn unless other factors such as the pH value of the medium, which may restrict all synthetic processes, are taken into consideration. It should be remembered that, when fungal growth on ammonium chloride almost stopped, there was an ample supply of sugar and nitrogen in the culture media.

Discussion

On comparing Figs. 1, 2, and 3, it can be noted that the rate of growth as measured by the dry weight increase was dependent on the rate of nitrogen absorption in the early period of growth (first to the fourth day) and on the rate of sugar uptake during the later growth period. In the first 4 days of incubation, nitrogen absorption was biggest from the nitrate media and smallest from the ammonium media. This was consequently reflected in the trend of the dry weight increase in the mycelial felts. In the later period of growth, the dry weight increase followed the sugar supply; it was highest in the asparagine cultures and lowest in the ammonium cultures.

On the other hand, when results of protein and fat contents of the mycelial felts are examined (Figs. 5 and 6), it appears quite clearly that there was an early and definite phase of growth during which there was a high rate of protein

synthesis and an extremely slow rate of fat synthesis. This took place in the first 4 days of incubation on the three different nitrogen sources. Later, in nitrate and asparagine cultures, protein synthesis slowed down while active fat synthesis started, more so on asparagine than on nitrate, and continued until the media were depleted of their sugar content. The dry weight, the fat content, and also the total carbohydrate (Fig. 4) all reached their maximum, in either the nitrate or asparagine cultures, by the same time interval. It may be assumed, therefore, that real growth, which means actual increase in the protoplasmic constituents, occurred during the early incubation period when nitrogen was available in the culture media. When nitrogen became deficient, the big sugar supply which was still present in the external media flowed into other metabolic pools, namely synthesis of fat and carbohydrate. The increase in the dry weight of the mycelial felts during this later period was due to mere accretion of fats and carbohydrates and not to real growth. Hopton and Woodbine (11) working with seven *Hansenula* spp. have shown that when all the nitrogen has been utilized, fattening begins. They also found that while the cell number remains fairly constant, the weight increases in the second stage due to fat and polysaccharide accretion in a resting phase. Lindner (15) claimed that a protein generation and a fat generation can thus be demonstrated in the life of fat-forming microorganisms. It may also be interesting to mention in this respect that Witter and Stotz (33) grew *Fusarium lycopersici* on a full nutrient medium to get a good yield of cellular material (growth phase) and then replaced the old medium by a high-sugar, nitrogen-free medium to promote fat formation (metabolic phase). These two phases could be shown in the present work in both nitrate and asparagine cultures. In ammonium cultures, the fungus could proceed in growth through the first 4 days only. The mycelial felts at this stage had a high percentage of proteins exceeding even those of the asparagine and nitrate cultures (Table I). After this interval, the dry weight remained almost constant until the end of the experimental period in spite of the fact that a high sugar content (12%) and an ample supply of nitrogen (about 55 mg/100 ml) were still remaining in the culture media. No nitrogen was absorbed during the later period of incubation and nitrogenous compounds were rather escaping out of the mycelial cells. Sugar absorption continued very slowly probably just to maintain a low rate of respiration.

The inability of the fungus to continue growth on ammonium chloride may be attributed to the big drop in the pH value of the medium. It was found to reach 1.5 during the leveling off of the growth curve, a value too low to support normal growth. When samples were withdrawn from the incubator and sodium hydroxide was aseptically added to the culture flasks, growth was resumed and a big yield of mycelium could be obtained.

The restricted growth of fungi on ammonium salts of strong acids in general has been the experience of several workers such as Talley and Blank (31), Brian and co-workers (3), and Philip and Walker (20). It was possible in most cases to correlate this restricted growth with the big drop in the pH value of the culture media which commonly occurs consequent upon the

preferential absorption of the ammonium ions. Many investigators have reported the drop in the pH value of fungal cultures supplied with ammonium chloride, nitrate, or sulphate (Haskins and Weston (9) with *Karlingia rosea*, Darby and Mandels (7) with *Myrothecium verrucaria*, Shirakawa (24) with *Diplocarpon rosae*, and Barnett and Lilly (1) with *Choanephora cucurbitarum*). It may be interesting to mention that Craseman (6) could help *Chytridium* to utilize ammonium nitrogen from ammonium sulphate as nitrogen source by daily neutralization of the culture media. It seems therefore that no conclusions as regards the inability of *Aspergillus nidulans* to utilize the ammonium nitrogen can be given until further trials are undertaken such as the use of ammonium salts of weak acids as carbonate or phosphate, or the application of an effective buffer system in the culture media.

The present findings also show that, under the present experimental conditions, asparagine was by far more conducive to fat formation than was sodium nitrate. On the contrary to this, a higher carbohydrate content was found in mycelial felts fed with nitrate in contrast to those fed with asparagine. The enzymatic systems in mycelial felts of *Aspergillus nidulans* grown on asparagine seem therefore to operate in directing the sugar supply towards synthesis of fat rather than of carbohydrates. Urea was found better than sodium nitrate for fat formation by *Penicillium spinulosum* but less favorable in *Penicillium javanicum* (Garrido and Walker (8)).

Synthesis of proteins was found to be higher from nitrate than from asparagine (Fig. 5). On ammonium chloride, the total protein content was relatively low, but calculated on percentage dry weight basis, proteins were highest in ammonium chloride cultures. A parallel conclusion was obtained by Subramanian and Pai (30) in their work with *Fusarium vasinfectum*. The restricted fungal growth on ammonium nitrogen in the present work did not show the real attitude of the fungus towards ammonium utilization, which may be clarified if it is given the chance to flourish upon ammonium nitrogen. However, there are different conclusions as to synthesis of proteins from different nitrogen sources. While more proteins are formed from ammonium than from nitrate in sweet potato tubers (Shishiny (25)), and in tomato plants (Clark(4)), Sideris and co-workers (26) found that pineapple plants build more protein from nitrate than from ammonium nitrogen.

Acknowledgment

The authors wish to express their thanks to Professor H. Saïd, Head of the Botany Department, Faculty of Science, Cairo University, for his kind interest in the work.

References

1. Barnett, H. L. and Lilly, V. G. 1956. Factors affecting the production of zygospores by *Choanephora cucurbitarum*. Mycologia, **48**, 617–627.
2. Bichkovskaya, A. L. 1940. The formation of fat by the fungus *Endomyces vernalis*. I. The influence of the composition of the substrate on the formation of fat by *E. vernalis* under conditions of film growth. Microbiology (Moscow), **8**, 1170–1180; Chem. Abstr. **42**, 5850 (1942).

3. BRIAN, P. W., CURTIS, P. J., and HEMMING, H. G. 1947. Glutinosin, a fungistatic metabolic product of the mould *Metarrhizium glutinosum* S. Pope. Proc. Roy. Soc. (London), B, **135**, 106–132.
4. CLARK, H. E. 1936. The effect of ammonium and nitrate nitrogen on the composition of the tomato plant. Plant Physiol. **11**, 5–24.
5. COCKRANE, V. W. 1958. Physiology of fungi. John Wiley & Sons, Inc., New York. p. 58.
6. CRASEMAN, J. M. 1954. The nutrition of *Chytridium* and *Macrochytridium*. Am. J. Botany, **41**, 302–310.
7. DARBY, R. T. and MANDELS, G. R. 1954. Inorganic nutrition of *Myrothecium verrucaria*. Mycologia, **46**, 276–288.
8. GARRIDO, J. M. and WALKER, T. K. 1956. Mycological formation of fat. II. Synthesis of fat from various carbohydrates in surface cultures of *Aspergillus nidulans*, *Penicillium javanicum*, and *Penicillium spiulosum* and the influence of the nitrogen source on the synthesis of fat from glucose. J. Sci. Food Agr. **4**, 233–237.
9. HASKINS, R. H. and WESTON, W. H. 1950. Studies in the lower Chytridiales. I. Factors affecting pigmentation, growth and metabolism of a strain of *Karlingia* (*Rhizophlictis*) *rosea*. Am. J. Botany, **37**, 739–750.
10. HEIDE, S. 1939. Zur Physiologie und Cytologie der Fattbildung bei *Endomyces vernalis* mit einen Beitrage zur Methodik quantitativen Bestimmung kleinster Fettmengen. Arch. Mikrobiol. **10**, 135–188.
11. HOPTON, J. W. and WOODBINE, M. 1958. Microbial fats: Comgrative assessment of some *Hansenula* spp. of yeasts. Abstr. Intern. Congr. Microbiol. 7th Congr. P365.
12. KAIBARA, T. 1948. Fat production by mold I. J. Agr. Chem. Soc. Japan, **22**, 89–90.
13. KLEINZELLER, A. 1948. Synthesis of lipides. Advances in Enzymol. **8**, 299–341.
14. LILLY, V. G. and BARNETT, H. L. 1951. Physiology of the fungi. McGraw-Hill Book Company, Inc., New York. p. 121.
15. LINDNER, P. 1922. Das Problem der biologischen Fettbildung und Fettgewinnung. Z. angew. Chem. **35**, 110–114.
16. LOCKWOOD, L. B., WARD, G. E., MAY, O. E., HERRICK, H. T., and O'NEILL, H. T. 1934. The production of fat by *Penicillium javanicum* van Beyma. Zentr. Bakteriol. **90**, 411–425.
17. NAGUIB, K. 1959. Growth and metabolism of *Aspergillus nidulans* Eidam in surface culture. Can. J. Botany, **37**, 353–364.
18. NAGUIB, K. 1959. The use of acetate in the metabolism of *Penicillium lilacinum* Thom. particularly in relation to synthesis of fat. Arch. Mikrobiol. **34**, 319–324.
19. NAGUIB, K. and WALKER, T. K. 1958. Microbiological synthesis of fat. The effects of nitrogen level on the metabolism of *Penicillium lilacinum* Thom. in a sucrose medium. J. Exptl. Botany, **9**, 109–114.
20. PHILIP, S. E. and WALKER, T. K. 1958. Mycological formation of fat. V. Factors which influence the formation of fat in surface cultures of *Penicillium lilacinum* Thom. J. Sci. Food Agr. **9**, 223–227.
21. PRILL, E. A., WENCK, P. R., and PETERSON, W. H. 1935. The chemistry of mold tissue VI. Factors influencing the amount and nature of fat produced by *Aspergillus fischeri*. Biochem. J. **29**, 21–33.
22. REICHEL, L. and REINMUTH, W. 1938. Fat formation from carbohydrates by *Endomyces vernalis*. Biochem. Z. **299**, 359–362; Chem. Abstr. **33**, 3415 (1939).
23. ROBBINS, W. J. 1937. The assimilation by plants of various forms of nitrogen. Am. J. Botany, **24**, 243–250.
24. SHIRAKAWA, H. S. 1955. The nutrition of *Diplocarpon rosae*. Am. J. Botany, **42**, 379–384.
25. SHISHINY, E. D. H. 1955. Absorption and assimilation of inorganic nitrogen from different sources by storage root-tissue. J. Exptl. Botany, **6**, 6–16.
26. SIDERIS, C. P., KRAUSS, B. H., and YOUNG, H. Y. 1938. Assimilation of ammonia and nitrate by pineapple plants growing in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. Plant Physiol. **13**, 489–527.
27. STEINBERG, R. A. 1939. Growth of fungi in synthetic nutrient solutions. Botan. Rev. **5**, 327–350.
28. STEINBERG, R. A. 1950. Growth of fungi in synthetic nutrient solutions. II. Botan. Rev. **16**, 208–228.
29. STREET, H. E., KENYON, A. E., and WATSON, G. M. 1946. The assimilation of ammonium and nitrate nitrogen by detached potato sprouts. Ann. Appl. Biol. **33**, 369–381.
30. SUBRAMANIAN, C. V. and PAI, K. V. S. 1953. Relation of nitrogen to growth and sporulation of *Fusarium vasinfectum* Atk. Proc. Indian Acad. Sci. **37**, 149–157.
31. TELLEY, P. J. and BLANK, L. M. 1942. Some factors influencing the utilization of inorganic nitrogen by the root rot fungus. Plant Physiol. **17**, 52–68.
32. TAMIYA, H. 1942. Atmung, Gärung und die sich daran beteiligenden Enzyme von *Aspergillus*. Advances in Enzymol. **2**, 183–238.
33. WITTER, R. F. and STOTZ, E. 1946. Fat formation in *Fusarium lycopersici*. Arch. Biochem. **9**, 331–339.

IODOACETATE INHIBITION OF PHOTOSYNTHETIC CARBON DIOXIDE ASSIMILATION IN SUGAR BEET AND SOYBEAN LEAVES[1]

D. C. MORTIMER

Abstract

When the petioles of freshly excised sugar beet or soybean leaves were immersed in dilute iodoacetate solutions, the rates of photosynthetic carbon dioxide assimilation were reduced and characteristic changes in the distribution of assimilated C^{14} were observed. Inhibited sugar beet leaves accumulated C^{14} in phosphoglyceric acid (PGA) and aspartic acid, but did not incorporate C^{14} into glyceric acid. Inhibited soybean leaves continued to form glyceric acid, but did not incorporate C^{14} into PGA and only trace amounts into aspartic acid. Incorporation of C^{14} into sucrose by the sugar beet leaf ceased when the assimilation of carbon dioxide was reduced below 1/10th of normal, while soybean leaves still formed sucrose at that level of inhibition. It was concluded from these and other data that the mechanisms for photosynthetic carbon dioxide assimilation in the two species were different. The iodoacetate-sensitive, PGA-glyceraldehyde-3-phosphate reduction, which could account for only part of the assimilation by sugar beet leaves, did not appear to be utilized by soybean leaves. An alternate pathway for the reduction of carbon dioxide to the carbohydrate level is postulated to account for the assimilation patterns observed in the two species.

Introduction

Considerable evidence supports the theory that the initial stages of photosynthetic carbon dioxide assimilation involve the carboxylation of ribulose diphosphate followed by hydrolysis to 2 moles of phosphoglyceric acid (PGA) (3, 8, 15, 18, 21). The reduction of PGA to the triose level is presumably catalyzed by one or more of the glyceraldehyde-3-phosphate dehydrogenases which have been isolated from leaves (1, 9). Although these enzymes, in vitro, show the appropriate sensitivity toward iodoacetate and iodoacetamide (1, 6), in the intact plant results seem less conclusive. Calvin and co-workers (5) found that although total carbon dioxide assimilation by *Chlorella* was markedly reduced in the presence of iodoacetate, an increased proportion of assimilated carbon was recovered in sucrose, and PGA did not accumulate. Newburgh and Burris (17) observed a decrease in the proportion of C^{14} fixed in PGA during photosynthesis by iodoacetate inhibited tobacco leaves. However, Arnon reported (1) that *Chlorella* cells showed a significant accumulation of PGA and a decrease in sucrose synthesis only when the cells were preincubated for a relatively long time in iodoacetate. Fraser (7) likewise observed that about one hour preincubation produced maximum inhibitory effects with *Chlorella*. Simonis and Weichert (19) studied effects of iodoacetate inhibition on the distribution of P^{32} assimilated by *Elodea*, and concluded that PGA was reduced to the triose level during photosynthesis.

[1]Manuscript received March 31, 1960.
Contribution from the Division of Applied Biology, National Research Council, Ottawa, Canada. Presented in part at the Ninth International Botanical Congress, August 19–29, 1959, Montreal, Canada.
Issued as N.R.C. No. 5714.

This report presents data obtained by allowing detached leaves of sugar beet and soybean to absorb iodoacetate solutions of different concentrations through the petiole either during or just prior to photosynthetic assimilation of $C^{14}O_2$ by the leaves. The inhibitory effects of iodoacetate on total carbon dioxide assimilation and on products resulting from the assimilation show that extensive pre-equilibration with the inhibitor was not necessary and that the two species responded differently to the inhibitor. The results are presented in support of the view that an alternate pathway for carbon assimilation during photosynthesis exists in some plants.

Experimental

Sugar beet plants (*Beta vulgaris* var. Klein Wanzleben) were grown in the greenhouse on nutrient-fed washed sand (4). Soybean plants (*Glycine max* var. Comet) were grown in a growth chamber maintained at 21.5° C, on coarse exploded mica (Vermiculite) in pots irrigated from below with tap water or a diluted nutrient solution similar to that used with the sugar beets. Illumination at the leaf surface was 2000 ft-c, supplied by water-filtered tungsten-filament lamps for 16 hours per day. Leaves from both species were excised by cutting under water and used immediately. All experiments were done between 9:00 and 10:30 a.m.

Freshly prepared iodoacetic acid solutions were titrated to pH 5.5 to 6.5 with sodium hydroxide and diluted to the appropriate concentration. The sugar beet leaf petiole was placed in a 20-ml beaker containing water, and its length was reduced to 2 cm by cutting under water. This leaf–beaker unit was moved into the fume hood under a source of heat-filtered, tungsten-filament illumination providing 2000 ft-c at the leaf surface. After 5 minutes, the water in the beaker was replaced by the iodoacetate solution, with care being taken to avoid exposing the cut end of the petiole to air. The leaves (2.5–3.0 g fresh weight) took up iodoacetate solution and water at the rate of 1 ml per hour when the leaf was illuminated in the draft of the fume hood. With 0.02 *M* iodoacetate, this rate was equivalent to the absorption of 0.3 μmoles of iodoacetate/minute. Any leaf which did not remain fully turgid during the uptake period was discarded. After 5, 10, or 15 minutes, the leaf was transferred to a photosynthesis bag without removing it from the illumination area. The bag was flattened to express excess air, sealed, and 100 ml of air containing 100 μc of $C^{14}O_2$ was introduced from a large syringe. After 30 or 60 seconds' photosynthesis in this atmosphere, the leaf was removed from the bag, one section was cut off and dropped into liquid nitrogen, the remainder was cut into an appropriate number of pieces and held in the light or in the dark or in combinations of both for different times before immersion in liquid nitrogen. Soybean trifoliate leaves were handled in the same way. Solution uptake by the soybean leaves (1 g) was considerably smaller than that by sugar beet leaves. Leaves which showed appreciable photonastic collapse were not used. The individual leaflets were the samples used in time sequence studies.

Methods for extracting the leaf parts, chromatography of the extracts, and analysis of the radioactivity distribution among the products of photosynthesis were the same as previously used (4).

The effect of iodoacetate uptake on the rate of carbon dioxide assimilation was measured in the continuous-flow, closed-circuit assimilation apparatus (14). In this type of experiment, the petiole, about 5 cm long, extended through a special channel in the photosynthesis chamber into the beaker containing water or iodoacetate solution. A current of air was forced through the illuminated box during the uptake period. After the predetermined time had elapsed, the normal air was replaced by air containing a measured amount of $C^{14}O_2$. Since the total carbon dioxide in the atmosphere of the system was known, the rate of disappearance of radioactivity from the atmosphere provided a means of determining the rate of assimilation.

Results

When the petiole of a sugar beet leaf was immersed in iodoacetate solution, inhibition could be detected within a few minutes either by measurement of total carbon dioxide uptake or by changes in products of photosynthetic assimilation. The curves in Fig. 1 show the relative carbon dioxide assimilation by leaves which had taken up water or iodoacetate solution for a 5-minute period immediately preceding the assimilation measurements. After 10 minutes' photosynthesis in the apparatus, the control leaf (water uptake) had assimilated about 0.81 mg of carbon dioxide, while those treated with 0.004 and 0.02 *M* iodoacetate solution had assimilated 0.59 and 0.28 mg respectively.

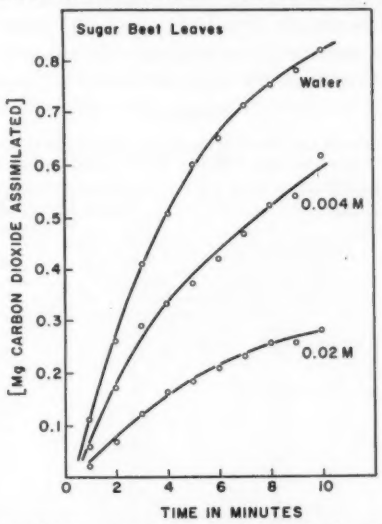


FIG. 1. Iodoacetate inhibition of carbon dioxide assimilation by detached sugar beet leaves in a closed system containing air. Petioles were immersed in water, 0.004 *M*, or 0.02 *M* iodoacetate solution for 5 minutes before zero time. Illumination throughout was 2000 ft-c. Total carbon dioxide at zero time was 1.0 mg.

If the "incubation" period in 0.02 *M* iodoacetate was extended beyond 5 minutes, carbon dioxide assimilation was further reduced. When the petiole was immersed in 0.02 *M* iodoacetate solution at the same time as assimilation measurements were started, that is, at time zero on the scale in Fig. 1, the reduction in assimilation was detected after about 3 minutes.

Control of the degree of inhibition with soybean leaves was not readily accomplished. With 0.01 *M* iodoacetate, inhibition was slight, while at 0.02 *M* there was almost complete inhibition. The intermediate concentration of 0.015 *M* usually reduced carbon dioxide assimilation to about 1/10th the control value but considerable variation in the degree of inhibition was the general rule. Measurements of the rate of assimilation did not, therefore, yield reliable data. The trend of results obtained indicated that carbon dioxide assimilation was a linear function of time, in contrast with the logarithmic function observed with the sugar beet leaf.

Preliminary experiments with sugar beet leaves revealed that the length of petiole attached to the leaf influenced the volume of iodoacetate solution absorbed. That is, 10 minutes of solution uptake through a petiole 3 to 5 cm long usually resulted in a gradient of iodoacetate inhibition upward through the leaf blade, while a similar uptake period through a petiole of 2 cm or less gave a relatively uniform inhibitory pattern throughout the whole leaf. These observations were based on the changes in distribution of C^{14} among the products of photosynthesis by leaves pretreated in the manner described. Transverse sections taken from different parts of the leaf were extracted and analyzed. Table I lists the percentage distribution of C^{14} among the products of 30 seconds' photosynthesis in a leaf treated to show "gradient" effects. The leaf was cut into five transverse sections, the tip, mid, and basal sections were analyzed, and the sections on either side of the mid section were discarded.

TABLE I

Influence of iodoacetate on the distribution of C^{14} among the ethanol-soluble products formed by the sugar beet leaf during 30 seconds' photosynthesis*

	Petiole in water for 10 min in the light	Petiole in 0.02 *M* iodoacetate for 10 min in the light		
		Tip section	Mid section	Basal section
	% of total ethanol-soluble C^{14}			
Hexose phosphates	23	43	15	21
Phosphoenolpyruvic acid	2	4	5	4
Phosphoglyceric acid	16	16	31	16
Glyceric acid	13	4	Trace	5
Aspartic acid	3	7	19	23
Malic acid	5	9	14	19
Glycine	10	4	0	0
Serine	18	8	6	7
Sucrose	6	2	5	5
Others	4	3	5	0
Relative C^{14} assimilation†	100	62	24	18

*Iodoacetate uptake through "long petiole". See text for details.

†The total C^{14} assimilated by each section of the iodoacetate-treated leaf was compared with that by the corresponding section of the water-treated leaf.

The control leaf received the same treatment throughout except that the petiole was immersed in water rather than iodoacetate solution. The results presented in Table I are from a single experiment rather than the average of those carried out to illustrate typical results. In the control leaf, the distribution of C^{14} among the products of photosynthesis was the same in tip, mid, and basal sections. In the iodoacetate-treated leaf, however, a decreasing carbon dioxide assimilation, and increasing proportions of assimilated C^{14} in aspartic and malic acids, were normally found in the tip-to-base direction. The formation of serine, glycine, and glyceric acid was markedly reduced in all sections and the lowest proportion of C^{14} in these compounds was usually in the base section. The increased proportion of C^{14} recovered in the hexose phosphate fraction and the decrease in sucrose observed in the tip section were not consistent features of this type of experiment. An increase in the proportion of C^{14} in PGA was found in most experiments, but as these results indicate, the increase did not bear a recognizable relation to the decrease in over-all carbon dioxide assimilation.

When iodoacetate solutions were offered to the sugar beet leaf through a short petiole a marked inhibition was often obtained with low iodoacetate concentration. For example, in Table II the uptake of 0.005 *M* iodoacetate for 10 minutes reduced the total assimilation to about 70% of the normal. In this case, the changes in distribution of C^{14} among products previously noted were accompanied by accumulation of C^{14} in PGA. The remaining columns in Table II give the C^{14} distribution observed after 60 seconds' photosynthesis by sugar beet leaves pretreated with different concentrations of iodoacetate solution for different intervals of time. The increases in proportion of C^{14} in aspartic and malic acids and the decreases in serine, glycine, and glyceric acid noted above were repeated throughout the series to differing degrees. With 0.02 *M* iodoacetate fed for 15 minutes, the total uptake of carbon dioxide was reduced to

TABLE II

Influence of iodoacetate on distribution of C^{14} among the ethanol-soluble products formed by sugar beet leaves during 60 seconds' photosynthesis*

	Iodoacetate concentration and duration of treatment					
	Control	0.005 *M*, 10 min	0.01 *M*, 10 min	0.01 *M*, 15 min	0.02 *M*, 5 min	0.02 *M*, 15 min
	% of total ethanol-soluble C^{14}					
Hexose phosphates	24	14	23	19	22	0
Phosphoenolpyruvic acid	3	2	2	1	5	0
Phosphoglyceric acid	15	40	19	21	32	22
Glyceric acid	10	3	3	1	0	0
Aspartic acid	3	15	15	21	10	23
Malic acid	9	8	13	21	11	49
Glycine	8	Trace	Trace	0	0	0
Serine	11	5	4	1	5	0
Alanine	4	3	2	3	7	5
Sucrose	12	8	15	10	8	0
Relative C^{14} assimilated	100	70	60	45	20	8

*Iodoacetate uptake through "short petiole". See text for details.

less than 10% of the control, and the only products still containing C^{14} were PGA, aspartic acid, malic acid, and alanine. The synthesis of sucrose was not completely blocked until the inhibition of total assimilation was in the order of 90% of the control. A similar series of experiments in which the photosynthesis period in $C^{14}O_2$ was reduced to 30 seconds gave results similar in every respect except for a slightly higher proportion of C^{14} in PGA.

The ultimate fate of the C^{14} assimilated during a 30-second photosynthetic period in subsequent photosynthesis and dark metabolism in normal air was followed by the technique previously described (4). The curves in Fig. 2 illustrate the changes in distribution brought about by the displacement treatment. Photosynthesis in normal air (Fig. 2*a*) resulted in a rapid depletion of the abnormally high proportion of C^{14} in PGA with a concurrent increase in the C^{14} in aspartic acid. Sucrose accumulated only a small part of the radioactivity in the iodoacetate-inhibited leaf in contrast to the pattern observed in the normal leaf (4). If the leaf was held in the dark immediately after the 30-second period of photosynthesis in $C^{14}O_2$ (Fig. 2*b*), the rapid loss of radioactivity from the PGA area was repeated, but alanine was now a major accumulant of C^{14} together with aspartic acid. When illumination was resumed the level of C^{14} in alanine fluctuated but remained relatively high, and sucrose did not accumulate C^{14}. In the normal leaf (4), this treatment caused a build-up of C^{14} in alanine during the dark period which was rapidly transferred to sucrose during the following light period.

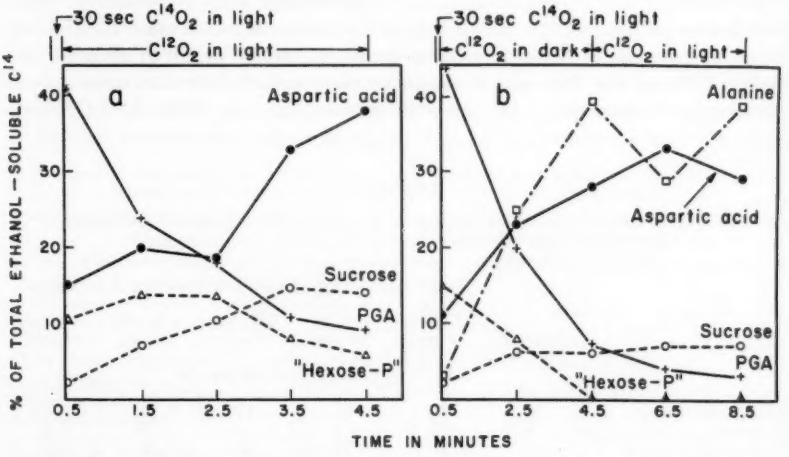


FIG. 2. Changes in distribution of C^{14} among the ethanol-soluble products formed by iodoacetate-inhibited sugar beet leaf in 30 seconds' photosynthesis, during subsequent displacement intervals in normal air. The displacement phase was carried out: (*a*) in continued illumination, or (*b*) in dark followed by illumination.

The leaf petiole was immersed in 0.01 *M* iodoacetate in the light for 10 minutes prior to the start of the experiment. Uptake of $C^{14}O_2$ was reduced to about 25% (*a*) and 10% (*b*) of normal.

In both leaves about 20% of the ethanol-soluble C^{14} was recovered in malic acid. Neither light nor dark displacement treatments altered this percentage significantly.

A similar series of iodoacetate inhibition studies was carried out with soybean trifoliate leaves. Although the uptake of carbon dioxide was reduced or almost completely blocked by iodoacetate solutions taken up through the petiole, this inhibition caused only minor changes in the distribution of C^{14} among the products of photosynthesis, as compared with the changes observed with the sugar beet leaf. Results shown in Table III were selected from a larger group of experiments as examples of the few in which changes in products were observed. In these, the inhibition reduced the total carbon dioxide uptake to 33% (10 minutes) and 7% (15 minutes) of the control. Most significant was the absence of any detectable accumulation of C^{14} in PGA. Sucrose formation was not appreciably reduced until the degree of inhibition was more than 90%. At this level, there was some increase in the proportion of C^{14} in malic and aspartic acids, and a decrease in serine and glycine, but there was no decrease in the radioactivity in glyceric acid or the hexose phosphate fraction.

TABLE III

Influence of iodoacetate on the distribution of C^{14} among the ethanol-soluble products formed by soybean leaves during 60 seconds' photosynthesis

		Iodoacetate concentration and treatment duration	
	Control	0.015 *M*, 10 min	0.015 *M*, 15 min
	% of total ethanol-soluble C^{14}		
Hexose phosphates	24	29	43
Glyceric acid	15	17	19
Aspartic acid	1	2	5
Malic acid	2	8	17
Glycine	7	6	0
Serine	10	10	3
Alanine	1	3	8
Sucrose	30	23	7
Relative C^{14} assimilated	100	33	7

When the displacement treatment was applied to iodoacetate-inhibited soybean leaves, there was little or no change in the distribution of radioactivity among the initial products during the 5-minute period of the experiments. In experiments where the degree of inhibition was less than 50%, the normal pattern of accumulation in sucrose was observed, although the rate of accumulation was less than that in the controls.

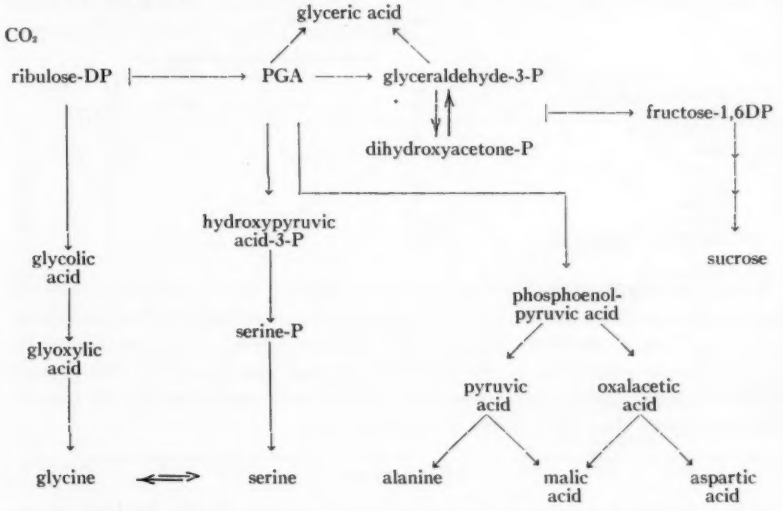
Discussion

Although the photosynthetic assimilation of carbon dioxide by both sugar beet and soybean leaves was reduced when the petioles were dipped in iodoacetate solutions, the two plants responded differently to the inhibitor. The level of inhibition in the sugar beet leaf could be readily controlled by changing the concentration of iodoacetate or the duration of the period of solution uptake. However, with soybean leaves, the effective range of inhibitor concentration and duration of uptake was very limited. The degree of inhibition in

replicate experiments could vary from very little to almost complete. In addition, the rate of carbon dioxide assimilation in the inhibited sugar beet leaf continued to follow normal carbon dioxide dependent first-order kinetics (14), while that in the treated soybean leaf, in addition to being difficult to measure, seemed to be controlled by factors other than the carbon dioxide concentration. If the mechanism for photosynthetic assimilation in these two species is the same, it is difficult to formulate a scheme by which the inhibitor could selectively block at different steps in the scheme in the two plants.

The rapidity with which the effects of the inhibitor could be detected were in marked contrast with the times recorded by Fraser (7) and Arnon (1) in their algal studies. Since the crystalline glyceraldehyde phosphate dehydrogenase was shown (6) to require up to 30 minutes' incubation with iodoacetate to achieve maximum inhibition, it is reasonable to assume that the observed inhibitions of carbon dioxide assimilation were, in the leaves, the resultant of several specific interactions, and not the blocking of a single specific enzyme reaction.

The pathways for formation of the compounds which become radioactive during short-term photosynthesis in sugar beet leaves are not fully established, but for the purposes of the present discussion, the scheme presented below has been drawn up.



This flowsheet, based on the review by Gibbs (10), gathers together various individual pathways between specific compounds which have been studied in several separate investigations. Assuming at the outset that iodoacetate blocks the transformation between PGA and glyceraldehyde-3-phosphate, we should predict that the C^{14} from photosynthetically assimilated carbon dioxide would accumulate in PGA. The increased C^{14} in PGA would result in

increased C^{14} in glyceric acid, serine, glycine, phosphoenolpyruvic acid, aspartic acid, malic acid, and alanine, and decreased C^{14} in sucrose. If the blockage were complete, glyceraldehyde-3-phosphate would not be formed and, hence, hexose phosphates and sucrose would not be formed. This scheme adequately accounted for some of the changes in distribution of C^{14} among the products of photosynthesis in iodoacetate-treated sugar beet leaves. However, since other changes in the sugar beet leaf products, and the absence of major changes in the products of soybean leaf photosynthesis, did not fit the indicated pathways, certain modifications will be suggested below.

The differences in response to iodoacetate inhibition by the two plant species, suggested in the over-all carbon dioxide uptake, was more clearly expressed when the formation of the different products of photosynthesis was considered. Because the alterations in distribution of C^{14} in the soybean leaf were limited even when the total carbon dioxide assimilation was reduced to 1/10th of normal, the extensive changes observed in the sugar beet leaf will be considered first, and each of the affected products will be considered separately.

Glyceric acid, normally regarded as a hydrolysis product of PGA, was not formed in the presence of moderate to high concentrations of iodoacetate, even though the expected accumulation of PGA did occur. It is unlikely, therefore, that the glyceric acid found in the normal leaf arises by phosphatase action prior to or during the extraction step. Its origin by way of glyceraldehyde-3-phosphate could account for the distinct reduction or complete inhibition observed, both in this study and in the previous cyanide inhibition investigation (13).

Glycine formation was more markedly inhibited by the iodoacetate than was serine formation. These observations are not consistent with the view that glycine is a precursor of serine, or that serine arises from PGA by way of hydroxypyruvic acid-3-phosphate. The second scheme for forming glycine and serine draws its precursor carbon from the ribulose pool (20), which substance should increase in concentration under the indirect influence of iodoacetate. The expected effect would then be to increase the levels of glycolic acid and glycine, and presumably serine. Since none of these postulated results were observed, a third mechanism must be sought to account for the normal mode of formation of the active photosynthetic intermediates, serine and glycine. The data suggest only that the three-carbon precursor of serine is, or is derived from, glyceraldehyde-3-phosphate rather than PGA, and that glycine formation seems to be independent of serine formation. The observation that the C^{14} recovered in aspartic acid increased with the increase in PGA, and, more significantly, that the displacement of C^{14} from PGA (Fig. 2*a*) occurred with a concurrent increase in the C^{14} of aspartic acid, indicate that there is a pathway between these two substances in the iodoacetate-treated leaf. The probable intermediate, phosphoenolpyruvic acid, showed a brief period of increased radioactivity in a few of the displacement experiments, but the transition was too rapid to be observed consistently. If the inhibition

of glyceraldehyde phosphate dehydrogenase is to be evaluated in terms of the accumulation of incorporated C^{14} in PGA, it is necessary to include most of the C^{14} recovered in aspartic acid.

Alanine formation in the light in the inhibited leaf was no greater than in the normal leaf (4), and it increased in the same way as the normal during the following dark period (Fig. 2*b*). However, in the inhibited leaf, dark-formed alanine remained in the leaf during subsequent illumination, whereas in the normal leaf it would have been converted to sucrose by way of pyruvic acid, PGA, and the hexose phosphates (4). This result provides additional evidence that the glyceraldehyde phosphate dehydrogenase system was blocked.

The increase in the proportion of C^{14} recovered in malic acid with increased levels of inhibition may be explained by assuming that the carboxylation reactions leading to malic acid are relatively insensitive to iodoacetate inhibition, and that formation of the acid continues in the inhibited leaf with little interference. The displacement experiments showed that the accumulation of incorporated C^{14} in aspartic acid had little, if any, influence on the C^{14} in malic acid. These results confirm previous indications (16) of independent routes of synthesis for malic and aspartic acids.

Sucrose formation continued in the iodoacetate-inhibited sugar beet leaf until the concentration of iodoacetate was high enough to prevent formation of the compounds labelled "hexose phosphates". The displacement experiments revealed a limited accumulation of C^{14} in sucrose which was approximately equal to the loss of radioactivity from the hexose phosphate fraction. If the scheme shown above is accepted, the observed formation of hexose phosphates and sucrose in the presence of iodoacetate indicates that part of the glyceraldehyde-3-phosphatase activity was not blocked by the inhibitor. The data, however, indicate the need for a more complex explanation. Firstly, the accumulation of assimilated C^{14} in PGA and the diversion of increasing amounts into aspartic acid with increasing degrees of inhibition should reduce the proportion of C^{14} reaching the hexose phosphates and sucrose. Secondly, the fact that alanine was not metabolized in the light portion of the displacement experiments suggests that its conversion to carbohydrate by way of PGA and glyceraldehyde-3-phosphate was blocked. These inhibition patterns may be explained by assuming that part of the photosynthetically assimilated $C^{14}O_2$ does not pass through the iodoacetate-sensitive, PGA–glyceraldehyde-3-phosphate reduction step, but undergoes reduction on the pathway to sucrose in a "by-pass" step which is relatively insensitive to iodoacetate. The general complexity of these changes observed in the sugar beet leaf, contrasted with the apparent immunity of the soybean carbon cycle to iodoacetate inhibition, serves to underline the possibility of different routes for carbon dioxide reduction.

Although carbon dioxide assimilation by soybean leaves was markedly reduced by iodoacetate, there were no notable changes in the distribution of carbon among the products. In particular, the absence of accumulation of radioactivity in PGA and aspartic acid suggests that little, if any, of the

assimilation occurs by way of the PGA–triose system. The continued formation of glyceric acid in the treated soybean leaf would indicate that its precursor was derived from the breakdown of a hexose precursor. When the assimilation of carbon dioxide was reduced to less than 10% of normal, sucrose formation was reduced but not eliminated, one of the unstable components of the hexose phosphate fraction accumulated C^{14}, and glycine formation was blocked. Although the data do not indicate which reactions iodoacetate might be blocking, they do show that the accepted schemes for photosynthetic assimilation do not account for the observed distributions of carbon in the soybean leaf. This type of photosynthetic distribution, observed several years ago by Aronoff (2), and found in this laboratory in leaves of other species as well as in soybean, must represent an alternate pathway (13) for photosynthetic carbon dioxide assimilation. The first products of photosynthesis by this route remain unidentified, but their possible relation to the β- or γ-carboxy pentose identified by Moses and Calvin (15) may be found in unpublished work from this laboratory that hydrolysis of the substances eluted from the hexose phosphate area of chromatograms, depending on the hydrolytic procedure, produces a hexose or glyceric acid.

The differences in response to iodoacetate of the two plant species studied reflect, with little reservation, the existence of different biosynthetic pathways for photosynthetic assimilation. If the PGA–triose pathway is taken as a characteristic symbol of the Calvin pathway, then the soybean leaf pathway may be a model for the second pathway. The mode of photosynthetic assimilation in the sugar beet leaf is believed to be a hybrid of the two. In those experiments where the short-term products of photosynthesis do not include significant levels of PGA, the second pathway may be considered dominant. Short-term photosynthesis experiments with sugar cane would come in this category since Kortschak, Hartt, and Burr (12) reported finding little or no PGA. These authors concluded that an alternate pathway must be used by sugar cane leaves. The changes in distribution of C^{14} among the products of photosynthesis in atmospheres containing different carbon dioxide levels (14) are more readily accounted for by assuming that the Calvin component of assimilation operates more efficiently at a lower carbon dioxide concentration than the soybean component. Other problems related to the photosynthetic assimilation of carbon dioxide, the Gibbs–Kandler asymmetric distribution of C^{14} in hexoses (11), for example, may be interpreted with less compromise by utilizing the two pathway concept.

Acknowledgment

I wish to express my appreciation to Mrs. A. Matheson for her excellent technical assistance.

References

1. Arnon, D. I. 1952. The glycolytic cycle in the breakdown and synthesis of carbohydrates in green leaves. *In* Phosphorus metabolism. Vol. II. The Johns Hopkins Press, Baltimore, Md., U.S.A. pp. 67–81.
2. Aronoff, S. 1951. Metabolism of soybean leaves III. The organic acids produced in short-time photosynthesis. Arch. Biochem. Biophys. **32**, 237–248.

3. BASSHAM, J. A. and CALVIN, M. 1957. The path of carbon in photosynthesis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, U.S.A.
4. BURMA, D. P. and MORTIMER, D. C. 1957. The fate of $C^{14}O_2$ in the sugar beet leaf studied by displacement with $C^{13}O_2$. Can. J. Biochem. and Physiol. **35**, 835–843.
5. CALVIN, M., BASSHAM, J. A., BENSON, A. A., LYNCH, V. H., OUELLET, C., SCHOU, L., STEPKA, W., and TOLBERT, N. E. 1951. Carbon dioxide assimilation in plants. Symposia Soc. Exptl. Biol. **5**, 284–305.
6. CORI, G. T., SLEIN, M. W., and CORI, C. F. 1948. Crystalline D-glyceraldehyde phosphate dehydrogenase from rabbit muscle. J. Biol. Chem. **173**, 605–618.
7. FRASER, I. M. 1954. The point of the inhibitory action of the iodoacetyl radical on photosynthesis. Australian J. Exptl. Biol. Med. Sci. **32**, 49–56.
8. GAFFRON, H., FAGER, E. W., and ROSENBERG, J. L. 1951. Intermediates in photosynthesis: Formation and transformation of phosphoglyceric acid. Symposia Soc. Exptl. Biol. **5**, 262–283.
9. GIBBS, M. 1952. Triosephosphate dehydrogenase and glucose-6-phosphate dehydrogenase in the pea plant. Nature, **170**, 164–165.
10. GIBBS, M. 1959. Metabolism of carbon compounds. Ann. Rev. Plant Physiol. **10**, 329–378.
11. GIBBS, M. and KANDLER, O. 1956. Asymmetric distribution of C^{14} in sugars formed during photosynthesis. Proc. Natl. Acad. Sci. U.S. **43**, 446–451.
12. KORTSCHAK, H. P., HARTT, C. E., and BURR, G. O. 1957. Phosphoglyceric acid and photosynthesis in the sugarcane. Proc. Hawaiian Acad. Sci. **21**.
13. MORTIMER, D. C. 1958. Evidence for an alternate pathway in photosynthetic assimilation. Naturwissenschaften, **45**, 116–117.
14. MORTIMER, D. C. 1959. Some short-term effects of increased carbon dioxide concentration on photosynthetic assimilation in leaves. Can. J. Botany, **37**, 1191–1201.
15. MOSES, V. and CALVIN, M. 1958. The identification of carboxyketopentitol diphosphates as products of photosynthesis. Proc. Natl. Acad. Sci. U.S. **44**, 260–277.
16. NELSON, C. D. and KROTKOV, G. 1956. Metabolism of C^{14} amino acids and amides in detached leaves. Can. J. Botany, **34**, 423–433.
17. NEWBURGH, R. W. and BURRIS, R. H. 1954. Effects of inhibitors on the photosynthetic fixation of carbon dioxide. Arch. Biochem. Biophys. **49**, 98–109.
18. RACKER, E. 1955. Synthesis of carbohydrates from carbon dioxide and hydrogen in a cell-free system. Nature, **175**, 249–251.
19. SIMONIS, W. and WEICHART, G. 1958. Die Wirkung von monojodessigsaure auf die bildung von P-markierten phosphorylierten verbindungen in Heleodia densa. Z. Naturforsch. **13b**, 694–696.
20. TOLBERT, N. E. 1958. Secretion of glycolic acid by chloroplasts. Brookhaven Symposia in Biol. **11**, 271–275.
21. WEISSBACH, A., HORECHER, B. L., and HURWITZ, J. 1956. The enzymatic formation of phosphoglyceric acid from ribulose diphosphate and carbon dioxide. J. Biol. Chem. **218**, 795–810.

A STUDY OF THE DISTRIBUTION OF CARBON-14 LABELLED COMPOUNDS IN STEM RUST INFECTED WHEAT LEAVES[1]

DALTON WANG

Abstract

Glucose-U-C^{14}, glycine-2-C^{14}, benzimidazole-2-C^{14}, as well as $C^{14}O_2$, were fed to rusted leaves of Little Club wheat. Radioautographs of whole leaves gave similar results to those of previous investigators, indicating greater radioactivity in rusted tissue sections. However, when quantitative measurements were made the results indicated that rusted tissue sections do not contain more radioactivity than the healthy tissue sections despite the apparent accumulation of labelled compounds at infection sites indicated by radioautographs of the treated leaves. The apparent accumulation of radioactivity at the infection sites of rusted leaves may not, therefore, represent the actual concentration of the labelled compounds throughout the infected leaf. The phenomenon of radioactivity accumulation indicated by radioautographs may be attributed to two factors: (*a*) the geometric factor in respect to radioactive sources to the X-ray film and (*b*) the specific distribution in respect to the higher radioactivity in the rapidly growing regions of the fungus.

Introduction

Gottlieb and Garner (5) first demonstrated the accumulation of radioactive phosphorus at the sites of rust infection. They found that the specific activity of the spores was less than that of either healthy or infected leaf tissues. The present author (13), however, found that urediospores collected from $KN^{15}O_3$-treated seedlings had a much higher N^{15} concentration than the host protein. Similarly, Staples and Ledbetter (11) showed that much more radioactivity of tritiated glycine was found in young urediospores. An accumulation of various labelled compounds at the sites of rust and powdery mildew infection was also reported by Shaw *et al.* (9, 10) and by Yarwood and Jacobsen (14, 15). On the whole, there is an agreement among these workers that an accumulation of fed chemicals takes place at the sites of infection of obligate and some facultative parasites. Mechanical injuries play only a minor role in accumulation. The accumulation at the infection site was attributed by Shaw and Samborski (10) to an active process. They suggested that the accumulation of fed chemicals took place in the infected or immediately adjacent host cells rather than in the parasite. Recently, Staples and Ledbetter (11), by means of microradioautographic technique, showed that much more radioactivity from glycine tritiated at 2-position was found in the young urediospores, their sporophores, and the distal portions of the fungal mycelia than in the host. Since the accumulation demonstrated by microradioautograph was mainly attributed to materials, such as protein and nucleic acids, which are insoluble in the organic solvents generally used in killing and fixing tissue sections, the amount of radioactivity in the compounds soluble in such organic solvents was undoubtedly neglected. This paper deals with the distribution of carbon-14 labelled compounds in the diseased leaves infected with *Puccinia graminis tritici*.

[1]Manuscript received in original form September 23, 1959, and, as revised, March 1, 1960.
Contribution from the Department of Botany, University of Manitoba, Winnipeg, Manitoba. This work was conducted under Extra Mural Research Grant No. 14 of the Canada Department of Agriculture.

Material and Methods

(1) Feeding Compounds Labelled with Carbon-14 to Wheat Leaves

Wheat plants of Little Club were inoculated with urediospores of *Puccinia graminis tritici* (race 15B-1) at the early two-leaf stage of greenhouse-grown plants and were then incubated in a moist chamber for 24 hours. At the end of incubation, they were placed on a bench in the greenhouse to allow rust to develop. Ten or more days later, the infected primary or secondary leaves were excised at the base with a razor blade. At that time the rust pustules were well developed, but with no secondary sporulation zone. The cut ends of 8–10 excised leaves were allowed to stand in a vial containing a solution of 0.1 ml of carbon-14 labelled compounds (with a total radioactivity of 1 μc) and 0.3 ml water and placed in a growth chamber (21° C) under illumination (650 ft-c). During the treatment, water was added after the solution had been absorbed and subsequently whenever necessary. The feeding period varied from 2 to 4 hours. In carbon-14 dioxide assimilation experiments, excised leaves with cut ends standing in a vial of water were placed in an atmosphere enriched with carbon-14 dioxide generated from $Na_2C^{14}O_3$ (2.5 μc) with sulphuric acid for a given period of time (from 15 to 30 minutes) in light (650 ft-c).

(2) Extraction and Determination of Radioactivity

At the end of the feeding or carbon-14 dioxide assimilation period, leaves were separated into rusted and healthy sections. For each section of rusted tissue two healthy ones, one above and below it, were usually taken. The leaf sections were placed in a weighing bottle and the fresh weight immediately determined. Boiling 85% ethanol was used for inactivating enzymes in the leaf tissues which were then refluxed for 30 minutes each with 80% and 40% ethanol respectively. The extracts were combined and evaporated *in vacuo* to dryness. The residue was dissolved in 80% ethanol and made to a final volume of 1 ml. Aliquots of 10 μl were taken and plated on planchets in duplicate. The radioactivity was determined with a thin mica end-window G-M counter connected to a Philips predetermined scaler (model PW 4035).

The remaining alcohol extract was quantitatively transferred and passed through Amberlite IR 120 and IR 45 resins. Amino acids and organic acids were retained on the columns, while sugars passed through in the effluent. Dilute ammonium hydroxide solution (1 *N*) was used as the eluting solvent for amino acids. The eluants from the two columns were combined. Thus two fractions, amino acids (including organic acids) and sugars, were obtained. Paper chromatograms and radioautographs were prepared according to the methods described below.

The extracted tissues (alcohol-insoluble fraction) were hydrolyzed with 6 *N* HCl in a sealed Pyrex glass test tube for 48 hours at 100° C. The hydrolyzate was centrifuged and the supernatant liquid including washings was transferred into a boiling flask and evaporated to dryness *in vacuo*. To remove HCl, evaporation of the hydrolyzate to dryness was repeated several times by

adding water to it. The hydrolyzate was dissolved in water and made up to a final volume of 1 ml. Ten-microliter aliquots of the hydrolyzate were taken for the determination of radioactivity.

(3) Paper Chromatography

Paper chromatography was carried out in an ascending manner on Whatman No. 1 paper with phenol:water (80:20 w/v), butanol:acetic acid:water (4:1:5 v/v), phenol saturated with pH 12.0 buffer (equal volume of 0.067 *M* Na_2HPO_4 and 0.067 *M* NaOH), or *m*-cresol saturated with pH 2.0 buffer (5 ml of 0.067 *M* KCl and 10.66 ml of 0.067 *M* HCl) as developing solvents for amino acids; and butanol:acetic acid:water (4:1:5 v/v), ethyl acetate:pyridine:water (2:1:2 v/v), or butanol:pyridine:water (45:25:40 v/v) as developing solvents for sugars. Cochromatography with known compounds was used for the identification of radioactive compounds.

(4) Radioautography

Radioautographs were prepared by superimposing Kodak (No-Screen) X-ray film upon each paper chromatogram with exposure periods of from 1 to 2 weeks.

(5) Spraying Reagents

(a) For Amino Acids

(i) Three per cent ninhydrin in 95% ethanol (1).

(ii) The ninhydrin – cupric nitrate indicator consists of a solution of 25 ml of 0.2% ninhydrin in 50 ml of absolute ethanol, 10 ml of glacial acetic acid, and 2 ml of 2,4,6-collidine, and 1.5 ml of a solution of 1% cupric nitrate trihydrate in absolute alcohol (8).

(iii) Pasieka's method for phenylalanine (1).

(b) For Sugars

(i) Orcinol reagent for ketoheptoses (1).

(ii) One per cent resorcinol in alcohol:0.2 *N* HCl (1:1 v/v) (14).

(iii) Triphenyltetrazolium chloride spray for reducing sugars (1).

Experimental Results

Glucose-U-C^{14}, glycine-2-C^{14}, benzimidazole-2-C^{14}, as well as $C^{14}O_2$, were fed to rusted leaves of Little Club wheat. Figure 1 shows two representative radioautographs of primary and secondary leaves that had been fed with glucose-U-C^{14} (1 μc) for 3½ and 4 hours, respectively. These results indicate an apparent accumulation of radioactivity at infection sites and are similar to those of previous investigators (9, 10, 14, 15).

The results given in Table I, however, show the effect of rust infection on the distribution of radioactivity in alcohol-soluble and alcohol-insoluble fractions of healthy and rusted tissue sections from the same leaves. It is quite evident that the healthy tissue sections possess a much higher radioactivity than the rusted ones in both fractions whether the calculation is based on a per unit weight or per unit area of the leaf. These results are not in agreement with the apparent accumulation of radioactivity as shown on the radioautographs of

the leaves. On the other hand, the radioactivity of the alcohol-soluble fraction in the healthy tissue sections of secondary leaves was less than that in the rusted ones from the same leaves with the exception of the experiment with glycine-2-C^{14} (Table II). However, when the data of the experiment with glycine-2-C^{14} were calculated on the basis of per unit area, the rusted tissue sections possessed slightly higher radioactivity than the healthy ones. This difference is small and may not be significant.

Table III shows the distribution of radioactivity in alcohol-soluble and alcohol-insoluble fractions of healthy and rusted tissue sections from rust-infected

TABLE I

The distribution of radioactivity in alcohol-soluble and alcohol-insoluble fractions of healthy and rusted sections from rust-infected leaves of Little Club wheat

Compound fed	Feeding period (hr)	Leaf	Age of infection (day)	Fraction	Radioactivity (c.p.m./unit wt.) Healthy	Rusted	Difference, H − R
Glycine-2-C^{14}	3	Primary	10	Alc. sol.	77,200 (72,700)*	29,700	+47,600 (+43,000)
				Alc. insol.	15,000 (10,100)	400	+11,600 (+ 6,700)
Glycine-2-C^{14}		Primary	10	Alc. sol.	24,400 (34,400)	23,100	+ 1,300 (+ 1,300)
				Alc. insol.	12,500 (9,000)	7,800	+ 4,700 (+ 1,200)
Glucose-U-C^{14}	3	Primary	15	Alc. sol.	32,000	27,700	+ 4,300
Glucose-U-C^{14}	3½	Primary	12	Alc. sol.	47,500	15,000	+32,500
				Alc. insol.	5,400	1,800	+ 3,600
Benzimidazole-2-C^{14}	3	Primary	10	Alc. sol.	99,900 (99,300)	29,600	+70,200 (+69,700)

*Figures in parentheses represent c.p.m./unit area of leaf sections. The weight in grams and the total area in centimeters of rusted leaf sections were adopted as a unit weight and a unit area, respectively.

TABLE II

The distribution of radioactivity in alcohol-soluble fractions of healthy and rusted sections from rust-infected leaves of Little Club wheat

Compound fed	Feeding period (hr)	Leaf	Age of infection (day)	Fraction	Radioactivity (c.p.m./unit wt.) Healthy	Rusted	Difference, H − R
Glycine-2-C^{14}	4	Secondary	10	Alc. sol.	17,800 (16,100)*	16,500	+ 1,300 (− 400)
Glucose-U-C^{14}	2	Secondary	10	Alc. sol.	34,400	44,000	− 9,600
Glucose-U-C^{14}	4	Secondary	10	Alc. sol.	21,600	29,900	− 8,300

*Figures in parentheses represent c.p.m./unit area of leaf sections.

TABLE III

The distribution of radioactivity in alcohol-soluble and alcohol-insoluble fractions of healthy and rusted sections from rust-infected leaves of Little Club wheat

Compound fed	Feeding period (min)	Leaf	Age of infection (day)	Fraction	Radioactivity (c.p.m./unit wt.) Healthy	Rusted	Difference, H − R
$C^{14}O_2$	15	Primary	10	Alc. sol.	24,400	16,700	+ 7,700
				Alc. insol.	2,200	1,600	+ 600
$C^{14}O_2$	30	Primary	10	Alc. sol.	34,500	19,200	+15,300
				Alc. insol.	2,300	900	+ 1,400
$C^{14}O_2$	20	Secondary	12	Alc. sol.	33,900	9,000	+24,900

PLATE I

FIG. 1. Radioautographs of primary and secondary leaves of Little Club showing the apparent accumulation of radioactive isotopes at rust infection sites (the white spots). Both primary and secondary leaves (from right to left) were fed with glucose-U-C^{14} (1 μc) through their cut ends, and the feeding periods were 3½ and 4 hours respectively.

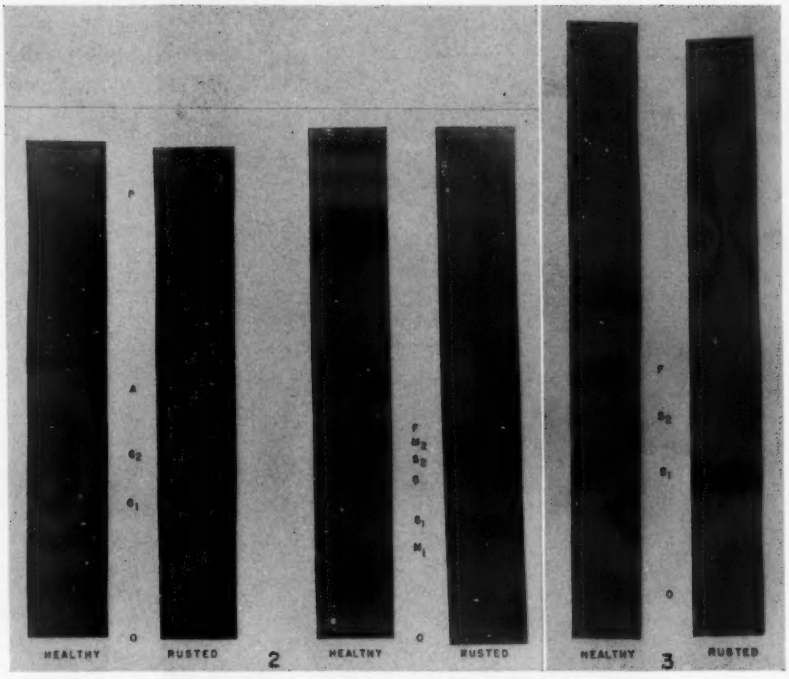


FIG. 2. Radioautographs of chromatograms of free amino acids and sugars (from left to right) in the healthy and rusted tissue sections from the same infected primary leaves of Little Club fed with glucose-U-C^{14} (1 μc), showing the distribution of carbon-14 in these compounds. A, G_1, G_2, P, and O represent alanine, glycine, glutamate, phenylalanine, and the origin respectively. F, G, M_1, M_2, S_1, S_2, and O represent fructose, glucose, maltose, mannose, sucrose, sedoheptulose, and the origin respectively. Developing solvent was butanol:acetic acid:water (4:1:5 v/v). Chromatograms were developed three times.

FIG. 3. Radioautographs of chromatograms of sugars, showing the distribution of carbon-14, in healthy and rusted tissue sections from the same infected primary leaves of Little Club exposed to $C^{14}O_2$ for 30 minutes. F, S_1, S_2, and O represent fructose, sucrose, sedoheptulose, and the origin respectively. Developing solvent was butanol:acetic acid: water (4:1:5 v/v). Chromatograms were developed three times.

leaves in the experiments of $C^{14}O_2$ assimilation. It is apparent that the radioactivity of both fractions from healthy tissue sections of primary leaves is much higher than that from rusted ones. Furthermore the experiment with secondary leaves gave results similar to those of primary leaves.

When rust-infected leaves were fed with glucose-U-C^{14}, a number of compounds became radioactive (Fig. 2). In the rusted regions of the tissue, almost half of the radioactivity in the sugar fraction was found in mannose. Sucrose was the second compound highly labelled, but no radioactive glucose was apparent. On the other hand, labelled glucose was still present in the healthy regions of the tissue and possessed the major portion of the isotope after 4 hours. However, it was found that healthy tissue sections are not always devoid of radioactive sucrose or rusted ones of labelled glucose. In amino acid fractions, only minor differences were evident.

In the $C^{14}O_2$ assimilation experiments, the labelling pattern of both amino acids and sugars was quite similar in healthy and rusted regions of the tissue. Sucrose was found to contain the highest radioactivity in both types of tissue sections (Fig. 3). In the healthy regions, sedoheptulose was found to contain a rather high concentration of the isotope, but little sedoheptulose was found in rusted parts. No radioactive mannose was evident in the $C^{14}O_2$ assimilation experiments.

Among the radioactive compounds, fructose, glucose, mannose, sedoheptulose, sucrose, maltose, alanine, glycine, glutamate, and phenylalanine were tentatively identified by cochromatography with known compounds and by their color complexes formed with specific reagents.

Discussion

Radioautographs of rust-infected leaves yielded results similar to those of previous investigators showing accumulation of radioactivity at infection sites (9, 10, 14, 15). However, the quantitative results of the present studies on the distribution of radioactivity in the infected leaves indicate that the healthy regions of primary leaves possess more isotope than the rusted regions. On the other hand, the healthy regions of secondary leaves from the feeding experiments had the same or somewhat smaller amount of radioactivity than the rusted sections (Table II). The discrepancy between primary and secondary leaves may be due to the difference in their physiological age, which may be the result of a greater rate of translocation in the latter, and may be also due to the fact that secondary leaves are narrower and a well-developed rust pustule may easily occupy the entire width of a leaf and thus exert greater interference upon the translocation stream. The latter is borne out by the following observations. In feeding experiments, where translocation is important in isotope spatial distribution throughout the leaf, the upper section adjacent to a pustule occupying the entire width of a secondary leaf always contained much less radioactivity than the corresponding upper section adjacent to a pustule occupying only a part of the leaf width. On the other hand, in $C^{14}O_2$ assimilation experiments where isotope distribution does not depend solely on upward

translocation there was little difference in the radioactivity of the upper and lower sections of leaves whether or not the pustule occupied a part or the whole width of the leaf. In these $C^{14}O_2$ experiments less radioactivity was also found in the rusted region as compared with the healthy region of the leaf. Nevertheless radioautograph showed an apparent accumulation of radioactivity at infection sites. This could be explained by the fact that at the infection site the epidermis is always ruptured by the massive rust sporophores and urediospores bulging out from the leaf surface. This could undoubtedly give a better contact with the X-ray film as well as a better chance for the β-particles emitted from the radioactive source to reach the X-ray film as compared with tissues with intact epidermis.

The apparent accumulation of radioactive isotope at infection sites is also possibly a result of the distribution of the mycelium in the wheat leaf and the specific distribution of the fed radioactive compound and its products in the mycelium. The distal parts of the rust mycelium tend to distribute themselves close to the epidermis on the pustule side. This would give the mycelium a geometric advantage with respect to the X-ray film provided radioactivity accumulated in the mycelium. It is highly possible that the actively growing regions of the fungus, which have a relatively high rate of metabolism, especially that of anabolism, possess the highest concentrations of the isotope. This idea is supported by the fact that urediospores collected from $KN^{15}O_3$-treated seedlings contained a much higher N^{15} concentration than the host protein (13) and that nearly half of the carbon-14 isotope in the sugar fraction is present in mannose (Fig. 2). This monosaccharide is apparently absent from healthy host tissues and presumably it exists in the fungus. Furthermore, Staples and Ledbetter (11) have reported that much radioactivity was found in the young urediospores, their sporophores, and the distal portions of the mycelium occupying the area which corresponds to zone III of Shaw and Samborski (10) for rusted leaves fed $C^{14}O_2$ in the light. This zone is a ring of radioactivity away from the center of infection.

Shaw and Samborski (10) have suggested that the inhibition of accumulation of glucose-1-C^{14} at infection sites of *Puccinia* or *Erysiphe* induced by azide, dinitrophenol, sulphur, and anaerobiosis is due to an inhibition of the process of active accumulation by the host tissue. However, sulphur has long been considered a fungicide for rust and mildew, and the inhibition of accumulation induced by this agent is most probably through its action on the fungus rather than on the host. Dinitrophenol blocks the utilization of the energy of oxidative reactions for synthetic purposes by inhibiting the phosphorylation that accompanies oxidative reactions. Thus, both dinitrophenol and anaerobiosis could interfere with the synthetic processes of the fungus and the host as well as the process of translocation. This interpretation and the experimental data reported in this paper underline the important role of fungal metabolic activity and growth of the fungus as well as the process of translocation in the apparent accumulation at infection sites.

The appearance of sucrose, which contained most of the radioactivity in the sugar fraction in the $C^{14}O_2$ assimilation experiments, is not unexpected, since

this compound has been reported to be the first free sugar to become labelled after only a few seconds of photosynthesis, while the free monosaccharides were devoid of label in as long as 4 minutes (2, 3, 4, 6). The presence of radioactive sucrose in the rusted tissue sections suggests that this compound may be the major sugar translocated, since little photosynthetic activity could have occurred in these sections. Sucrose was also reported to be translocated faster than fructose and glucose (12).

In view of the fact that the difference in the labelling pattern was mainly found in sugars between healthy and rusted tissue sections, it can be assumed that the fungus is capable of synthesizing some of its own required carbohydrates and does not depend entirely on the host for all its sugars. In the $C^{14}O_2$ assimilation experiments, the presence of labelled sedoheptulose essentially in the healthy regions of the rust-infected leaves gave unequivocal evidence that the accumulation of this labelled compound in rusted leaves of the susceptible plants (Little Club) reported by Jain and Pelletier (7) was taking place in the host tissues and not in the fungus.

In conclusion, evidence indicates that the apparent accumulation of radioactivity at the infection sites of rusted leaves shown on the radioautographs may not represent the actual distribution of the labelled compounds throughout the infected leaf. The phenomenon of the apparent accumulation of radioactive compounds at infection sites indicated by the radioautographs may be attributed to two major factors: (*a*) the geometric factor in respect to the radioactive sources to the X-ray film and (*b*) the specific distribution in respect to the higher radioactivity in rapidly growing regions of the fungus.

Acknowledgments

The author is grateful to Dr. E. R. Waygood, Head of the Department of Botany, University of Manitoba, Winnipeg, Manitoba, for his helpful criticism of the manuscript.

References

1. Block, R. J., Durrum, E. L., and Zweig, G. 1958. A manual of paper chromatography and paper electrophoresis. 2nd ed. Academic Press, Inc., New York.
2. Calvin, M. and Benson, A. A. 1949. Path of carbon in photosynthesis; the identity and sequence of the intermediates in sucrose synthesis. Science, **109**, 140–142.
3. Calvin, M., Bassham, J. A., and Benson, A. A. 1950. Chemical transformations of carbon in photosynthesis. Federation Proc. **9**, 524–534.
4. Gibbs, M. 1951. The position of C^{14} in sunflower leaf metabolites after exposure of leaves to short period photosynthesis and darkness in an atmosphere of $C^{14}O_2$. Plant Physiol. **26**, 549–556.
5. Gottlieb, D. and Garner, J. M. 1946. Rust and phosphorus distribution in wheat leaves. Phytopathology, **36**, 557–564.
6. Hassid, W. Z. and Putman, E. W. 1952. Sugar transformations in leaves of *Canna indica*. Federation Proc. **11**, 226–227.
7. Jain, A. C. and Pelletier, R. L. 1958. Effects of rust infection on the carbohydrate metabolism of wheat seedlings. Nature, **182**, 882–883.
8. Moffat, E. D. and Lytle, R. I. 1959. Polychromatic technique for the identification of amino acids on paper chromatograms. Anal. Chem. **31**, 926–928.
9. Shaw, M., Brown, S. A., and Rudd-Jones, D. 1954. Uptake of radioactive carbon and phosphorus by parasitized leaves. Nature, **173**, 768–769.

10. SHAW, M. and SAMBORSKI, D. J. 1956. The physiology of host–parasite relations. I. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. Can. J. Botany, **34**, 389–405.
11. STAPLES, R. C. and LEDBETTER, M. C. 1958. A study by microautoradiography of the distribution of tritium-labelled glycine in rusted Pinto bean leaves. Contribs. Boyce Thompson Inst. **19**, 349–354.
12. VERNON, L. P. and ARONOFF, S. 1952. Metabolism of soybean leaves. IV. Translocation from soybean leaves. Arch. Biochem. Biophys. **36**, 383–398.
13. WANG, D. 1957. Studies on the nature of resistance of plants to disease. Alterations in the nitrogen and keto acid metabolism of resistant and susceptible wheat varieties associated with infection by *Puccinia graminis tritici* Eriks. and Henn. Ph.D. Thesis, McGill University, Montreal, Que.
14. YARWOOD, C. E. and JACOBSEN, L. 1950. Selective absorption of sulfur-35 by fungus-infected leaves. Nature, **165**, 973–974.
15. YARWOOD, C. E. and JACOBSEN, L. 1955. Accumulation of chemicals in diseased areas of leaves. Phytopathology, **45**, 43–48.

THE TAXONOMY AND HABITATS OF FUSARIUM SPECIES FROM TROPICAL AND TEMPERATE REGIONS[1]

W. L. GORDON[2]

Abstract

The present taxonomic study involved approximately 1200 isolates of *Fusarium* which were received by the writer from time to time each year during the past 25 years from tropical and temperate regions. The various isolates originated in 59 geographic areas. Approximately 90% of the isolates were obtained from a total of 160 species of host plants which included cultivated and non-cultivated plants. The cultivated plants included the chief agricultural crops grown in tropical and temperate regions, respectively. About 10% of the isolates were obtained from miscellaneous habitats, e.g. soil, water, insects, wood pulp, fowl, dairy products, and human tissue.

A total of 26 species, 4 varieties, and 17 formae of *Fusarium*, classified in 12 sections of the genus, were identified among the isolates. These species, varieties, and formae are: *F. aquaeductuum* (Radlk. & Rabh. pr.p) Lagh., *F. dimerum* Penz., *F. merismoides* Cda. (section Eupionnotes); *F. decemcellulare* Brick (section Spicarioides); *F. nivale* (Fr.) Ces. (section Arachnites); *F. chlamydosporum* Wr. & Rg., *F. poae* (Cda.) Sacc., *F. sporotrichioides* Sherb. (section Sporotrichiella); *F. arthrosporioides* Sherb., *F. avenaceum* (Fr.) Sacc. (section Roseum); *F. semitectum* Berk. & Rav. (section Arthrosporiella); *F. acuminatum* (Ell. & Everh.) Wr., *F. compactum* (Wr.) Gordon, *F. equiseti* (Cda.) Sacc., *F. longipes* Wr. (section Gibbosum); *F. culmorum* (W. G. Sm.) Sacc., *F. graminearum* Schwabe, *F. heterosporum* Nees emend. Raillo; *F. sambucinum* Fuckel, *F. s.* var. *coeruleum* Wr., *F. s.* forma 6 Wr., *F. tumidum* Sherb. (section Discolor); *F. lateritium* Nees, *F. l.* forma *cajani* (Padw.) Gordon, *F. l.* forma *crotalariae* (Padw.) Gordon, *F. stilboides* Wr. (section Lateritium); *F. moniliforme* Sheld., *F. m.* var. *anthophilum* (A. Br.) Wr., *F. m.* var. *subglutinans* Wr. & Rg. (section Liseola); *F. oxysporum* Schl. emend. Snyder & Hansen, *F. oxysporum* var. *redolens* (Wr.) Gordon; *F. oxysporum* forma *callistephi* (Beach) S. & H.; forma *cepae* (Hanz.) S. & H., forma *dianthi* (Prill. & Del.) S. & H., forma *gladioli* (Massey) S. & H., forma *lini* (Bolley) S. & H., forma *lycopersici* (Sacc.) S. & H., forma *nicotianae* (Johns.) S. & H., forma *niveum* (E. F. S.) S. & H., forma *passiflorae* Purss, forma *pini* (Hartig) S. & H., forma *pisi* (Lindf.) S. & H., forma *tuberosi* S. & H., forma *vasinfectum* (Atk.) S. & H. (section Elegans); *F. coeruleum* (Lib.) Sacc., *F. solani* (Mart.) App. & Wr. emend. S. & H. (section Martiella).

As indicated by the number of geographic areas and habitats from which they were obtained, the six species most widely distributed were *F. solani*, *F. oxysporum*, *F. equiseti*, *F. moniliforme*, *F. avenaceum*, and *F. semitectum*. Five species, namely *F. aquaeductuum*, *F. chlamydosporum*, *F. arthrosporioides*, *F. longipes*, and *F. tumidum*, were encountered rarely, each being represented by a single isolate.

Introduction

Previous studies by the writer on the genus *Fusarium* have been confined chiefly to those species, varieties, and formae encountered in Canada (2, 3, 4, 5, 6, 7) and in Trinidad (8). As species of this genus are not only pathogenic to plants but also can exist as saprophytes within a wide range of environmental conditions and habitats, they have been encountered by others in many regions of the world. From time to time during each of the past 25 years

[1]Manuscript received April 22, 1960.
Contribution No. 53 from Canada Department of Agriculture Research Station, Winnipeg, Manitoba.
[2]Plant Pathology Laboratory.

the writer has received for identification isolates of *Fusarium* from many tropical and temperate regions. Most of these isolates were obtained by various research workers during the course of investigations on plant diseases in their respective geographic areas. Some had been shown to be pathogenic to the host plants from which they were obtained while others were suspected of being implicated in the disease condition. A small proportion of the total isolates received was obtained from miscellaneous habitats where their existence usually depended on their capabilities as saprophytes rather than on any potentialities as pathogens. These various isolates of *Fusarium* have formed the basis of the present taxonomic study which was carried on concurrently with those reported previously (2, 3, 4, 5, 6, 7, 8).

Approximately 1200 isolates of *Fusarium* were involved in this study. Most of these isolates were received by the writer from the regions in which they originated through the Commonwealth Mycological Institute, Kew, England. Others were received directly from individual research workers in the geographic areas where they were located. The various isolates of *Fusarium* originated in 59 geographic areas some of which were represented by many more isolates than others. The number of isolates obtained from each of these geographic areas is as follows:

Aden (1); Argentina (4); Australia (35); Belgian Congo (12); British Cameroons (1); British Honduras (1); Ceylon (4); Chile (1); Cyprus (56); Denmark (8); Egypt (84); England (87); Finland (6); France (3); French West Africa (1); Gambia (1); Ghana (19); Greece (14); Guernsey (3); Hebrides, Isle of Rhum (2); Honduras (13); India (84); Iran (2); Iraq (10); Israel (81); Jamaica (20); Kenya–Uganda (75); Malaya (10); Malta (3); Mauritius (3); Mexico (36); New Guinea (2); New Zealand (55); Netherlands (2); Nigeria (25); Northern Ireland (10); Northern Nigeria (4); Northern Rhodesia (10); Nyasaland (15); Pakistan (12); Portugal (85); Saint Helena (1); Sarawak (5); Scotland (54); Shetlands, Isle of Unst (1); Sierra Leone (9); South Africa (9); Southern Rhodesia (45); Spanish Guinea (1); Sudan (24); Sweden (1); Tanganyika (42); Tasmania (2); Thailand (1); Turkey (8); Uruguay (7); Venezuela (1); United States (91); and Wales (9).

Approximately 90% of the isolates of *Fusarium* included in this study were obtained from 160 species of plants both cultivated and non-cultivated and about 10% from miscellaneous habitats such as soil, water, insects, wood pulp, fowl, dairy products, and human tissue. The cultivated plants included the chief agricultural crops grown in tropical and temperate regions, respectively.

As this taxonomic study was carried on concurrently with others in which isolates of *Fusarium* from Canada (2, 3, 4, 5, 6, 7) and Trinidad (8) were involved the culture methods employed were those described previously (3). It is necessary to stress only that the various isolates were established as monosporous cultures on potato sucrose agar (2% agar, 2% sucrose) as the first step in the identification procedure.

Classification of the Isolates

The system of nomenclature and classification adopted in naming and classifying the various isolates is the same as that followed by the writer in previous studies (2, 3, 4, 5, 6, 7, 8). The system is basically that of Wollenweber and Reinking (14) modified where it appeared desirable to do so according to recent revisions of the genus (1, 9, 11, 12, 13).

According to the system of nomenclature and classification adopted, a total of 26 species, 4 varieties, and 17 formae of *Fusarium* were distinguished and identified among the isolates. These species, varieties, and formae of *Fusarium* represented 12 sections of the genus. These sections are Eupionnotes Wr.; Spicarioides Wr., Sherb., Rg., Johann, & Bailey; Arachnites Wr.; Sporotrichiella Wr.; Roseum Wr.; Arthrosporiella Sherb.; Gibbosum Wr.; Discolor, Wr.; Lateritium Wr.; Liseola Wr., Sherb., Rg., Johann, & Bailey; Elegans Wr.; and Martiella Wr.

The synonymy of the species, varieties, and formae of *Fusarium*, which is now voluminous, has been mostly omitted in this paper but can be obtained from the publications by Wollenweber and Reinking (14), Snyder and Hansen (11, 12, 13), Raillo (10), Bilai (1), and the writer (3, 5, 7, 8).

Section Eupionnotes Wr.

Wollenweber and Reinking, Die Fusarien, pp. 18–20. 1935.

Three species of *Fusarium*, namely *F. aquaeductuum* (Radlk. & Rabh. pr.p) Lagh., *F. dimerum* Penz., and *F. merismoides* Cda., belonging in this section of the genus, were identified among the various isolates.

FUSARIUM AQUAEDUCTUUM (Radlk. & Rabh. pr.p.) Lagh.
Wollenweber and Reinking, Die Fusarien, p. 20. 1935.
HABITAT: sewage treatment plant.

FUSARIUM DIMERUM Penz.
Wollenweber and Reinking, Die Fusarien, p. 26. 1935.
Gordon, Can. J. Botany, 30: 216. 1952.
HABITATS: *Elaeis guineensis* Jacq., *Malus sylvestris* Mill., *Musa paradisiaca* L. var. *sapientum* Kuntze, butter churn, sewage-bearing stream, and soil.

FUSARIUM MERISMOIDES Cda.
Wollenweber and Reinking, Die Fusarien, pp. 24–25. 1935.
Gordon, Can. J. Botany, 30: 216–217. 1952.
HABITATS: *Pyrus communis* L., effluent from creamery, cotton filters in a water filtering plant, and a sewage-bearing stream.

Section Spicarioides Wr., Sherb., Rg., Johann, & Bailey

Wollenweber and Reinking, Die Fusarien, p. 36. 1935.

This section of the genus was represented among the isolates by *Fusarium decemcellulare* Brick. It is usually encountered only in tropical and subtropical regions.

FUSARIUM DECEMCELLULARE Brick
Wollenweber and Reinking, Die Fusarien, p. 37. 1935.
Gordon, Can. J. Botany, 34: 850–851, 853. 1956.
HABITATS: *Anacardium occidentale* L., *Coffea arabica* L., *Coffea robusta* L. Linden, *Durio zibethinus* Murr., *Piper nigrum* L., *Theobroma cacao* L., and **Triticum aestivum* L.

*Denotes isolation from the seed of this host plant.

Section Arachnites Wr.

Wollenweber and Reinking, Die Fusarien, pp. 42–43. 1935.

This section of the genus was represented among the isolates by *Fusarium nivale* (Fr.) Ces. It is well known in temperate regions as a common cause of snow mold of Gramineae.

FUSARIUM NIVALE (Fr.) Ces.
Wollenweber and Reinking, Die Fusarien, p. 44. 1935.
Gordon, Can. J. Botany, 30: 217–218. 1952.
HABITATS: **Avena sativa* L., *Agrostis* sp., Gramineae, **Hordeum vulgare* L., and *Triticum aestivum* L.

Section Sporotrichiella Wr.

Wollenweber and Reinking, Die Fusarien, pp. 45–47. 1935.

Three species of *Fusarium*, namely *F. chlamydosporum* Wr. & Rg., *F. poae* (Pk.) Wr., and *F. sporotrichioides* Sherb., belonging in this section of the genus, were identified among the isolates.

FUSARIUM CHLAMYDOSPORUM Wr. & Rg.
Wollenweber and Reinking, Die Fusarien, pp. 47–48. 1935.
Gordon, Can. J. Botany, 37: 259–260. 1959.
HABITAT: soil.

FUSARIUM POAE (Pk.) Wr.
Wollenweber and Reinking, Die Fusarien, p. 47. 1935.
Gordon, Can. J. Botany, 30: 219. 1952.
HABITATS: *Citrus* sp., **Triticum aestivum* L., and sewage-water sediment.

FUSARIUM SPOROTRICHIOIDES Sherb.
Wollenweber and Reinking, Die Fusarien, pp. 48–49. 1935.
HABITATS: *Cynodon dactylon* Pers., *Hordeum vulgare* L., *Scirpus americanus* Pers., *Triticum aestivum* L., mange-like condition of a young pig, and sheep dung.

Section Roseum Wr.

Wollenweber and Reinking, Die Fusarien, pp. 49–53. 1935.

This section of the genus was represented among the various isolates by two species of *Fusarium*, namely *F. arthrosporioides* Sherb. and *F. avenaceum* (Fr.) Sacc.

FUSARIUM ARTHROSPORIOIDES Sherb.
Wollenweber and Reinking, Die Fusarien, pp. 56–57. 1935.
HABITAT: *Aleurites montana* Wils.

FUSARIUM AVENACEUM (Fr.) Sacc.
Wollenweber and Reinking, Die Fusarien, pp. 53–55. 1935.
Gordon, Can. J. Botany, 30: 221. 1952.
HABITATS: **Avena sativa* L., **Bromus marginatus* Nees, *Cytisus scoparius* Link., **Dactylis glomerata* L., *Dianthus caryophyllus* L., Gramineae, *Heracleum sphondylium* L., *Lathyrus odoratus* L., *Linum usitatissimum* L., *Malus sylvestris* Mill., *Muhlenbergia racemosa* (Michx.) B.S.P., *Paspalum dilatatum* Poir, *Pinus* sp., **Pisum sativum* L., *Rubus idaeus* L., *Solanum tuberosum* L., *Trifolium repens* L., *Triticum aestivum* L., *Vicia faba* L., **Vicia sativa* L., dairy products, and soil.

Section Arthrosporiella Sherb.

Wollenweber and Reinking, Die Fusarien, pp. 57–58. 1935.

Only one species of *Fusarium*, namely *F. semitectum* Berk. & Rav., belonging in this section of the genus, was identified among the isolates.

FUSARIUM SEMITECTUM Berk. & Rav.

Wollenweber and Reinking, Die Fusarien, pp. 58–59. 1935.
Gordon, Can. J. Botany, 30: 222–223. 1952.

HABITATS: *Arachis hypogaea* L. **Bombax malabaricum* DC., **Capsicum annuum* L., *Chrysanthemum cinerariaefolium* Vis., *Citrus aurantifolia* Swingle, *Citrus medica* L., *Citrus* sp., *Coffea arabica* L., *Coffea robusta* L. Linden, **Eclipta alba* (L.) Hassk., *Gossypium* sp., **Hibiscus cannabinus* L., *Lycopersicum esculentum* Mill., *Musa paradisiaca* L. var. *sapientum* Kuntze, *Piper betle* L., *Ricinus communis* L., *Solanum tuberosum* L., **Sorghum* sp., *Zea mays* L., parasitized grasshopper, stored sugar, orchard air, sewage treatment plant, and soil.

Section Gibbosum Wr.

Wollenweber and Reinking, Die Fusarien, pp. 61–63. 1935.

Gordon, Can. J. Botany, 30 : 223–224. 1952.

This section of the genus was represented among the isolates by the four species of *Fusarium* classified in it (3). These species are *F. acuminatum* (Ell. & Everh.) Wr., *F. compactum* (Wr.) Gordon, *F. equiseti* (Cda.) Sacc., and *F. longipes* Wr.

FUSARIUM ACUMINATUM (Ell. & Everh.) Wr.

Wollenweber and Reinking, Die Fusarien, pp. 67–68. 1935.
Gordon, Can. J. Botany, 30: 226. 1952.

HABITATS: *Beta vulgaris* L., *Dianthus caryophyllus* L., *Crotalaria juncea* L., *Linum usitatissimum* L., **Lolium perenne* L., *Lycopersicum esculentum* Mill., *Malus sylvestris* Mill., *Medicago sativa* L., *Picea morinda* Link, *Pinus caribaea* Morelet., *Schoenus nigricans* L., *Solanum tuberosum* L., *Vitis* sp., sewage treatment plant, and soil.

FUSARIUM COMPACTUM (Wr.) Gordon

Gordon, Can. J. Botany, 30: 224. 1952; 32: 580–582. 1954.

HABITATS: *Arachis hypogaea* L., *Lycopersicum esculentum* L., *Musa paradisiaca* L. var. *sapientum* Kuntze, *Pinus elliotti* Engelm., *Triticum aestivum* L., and soil.

FUSARIUM EQUISETI (Cda.) Sacc.

Wollenweber and Reinking, Die Fusarien, pp. 63–64. 1935.
Gordon, Can. J. Botany, 30: 225–226. 1952.

HABITATS: *Antirrhinum majus* L., *Arachis hypogaea* L., *Bothriochloa insculpta* (Hochst.) A. Camus, **Callistephus chinensis* Nees, *Carica papaya* L., **Chloris gayana* Kunth., *Chrysanthemum cinerariaefolium* Vis., *Citrus aurantifolia* Swingle, *Citrus limonia* Osbeck, *Citrus* sp., *Coffea* sp., *Crotalaria juncea* L., *Cucurbita pepo* L., *Cynodon dactylon* Pers., *Cynodon plectostachyum* Pilger, *Cytisus scoparius* Link, *Dahlia* sp., *Dianthus caryophyllus* L., *Eucalyptus* sp., *Gossypium* sp., *Helianthus annuus* L., *Hevea* sp., **Hordeum vulgare* L., *Lactuca sativa* L., *Linum usitatissimum* L., *Lolium perenne* L., *Lycoperscium esculentum* Mill., *Malus sylvestris* Mill., *Manihot utilissima* Pohl, *Medicago sativa* L., *Musa paradisiaca* L. var. *sapientum* Kuntze, **Ornithopus sativus* Brot., *Oryza sativa* L., *Passiflora edulis* Sims., *Pennisetum mairva* Staff. & Hubbard, *Phaseolus vulgaris* L., *Poinciana regia* Bojer, *Prunus armeniaca* L., *Solanum tuberosum* L., *Sorghum vulgare* Pers., *Sorghum* sp., *Theobroma cacao* L., *Triticum aestivum* L., *Vicia faba* L., *Zea mays* L., *Psalliota* sp., orchard air, termite mound, sewage treatment plant, and soil.

FUSARIUM LONGIPES Wr.

Wollenweber and Reinking, Phytopathology, 15: 155–169. 1925.
Gordon, Can. J. Botany, 30: 224. 1952.

Syn. *F. scirpi* Lamb. & Fautr. var. *longipes* (Wr. & Rg.) Wr.
Wollenweber and Reinking, Die Fusarien, pp. 68–69. 1935.

HABITAT: soil.

Section Discolor Wr.

Wollenweber and Reinking, Die Fusarien, pp. 69–72. 1935.

Gordon, Can. J. Botany, 30 : 227. 1952.

This section of the genus was represented among the isolates by five species, one variety, and one forma of *Fusarium*, namely *F. culmorum* (W. G. Sm.)

Sacc., *F. graminearum* Schwabe, *F. heterosporum* Nees emend. Raillo, *F. sambucinum* Fuckel, *F. sambucinum* var. *coeruleum* Wr., *F. sambucinum* forma 6 Wr., and *F. tumidum* Sherb.

FUSARIUM CULMORUM (W. G. Sm.) Sacc.

Wollenweber and Reinking, Die Fusarien, pp. 79–81. 1935.

HABITATS: *Cucurbita pepo* L., *Dianthus caryophyllus* L., *Gladiolus* sp., *Gossypium* sp., Gramineae, **Hordeum vulgare* L., *Lactuca sativa* L., *Linum usitatissimum* L., *Lolium perenne* L., *Medicago sativa* L., *Morus nigra* L., *Rubus idaeus* L., *Solanum tuberosum* L., **Triticum aestivum* L., *Zea mays* L., hens' eggs (shell membranes with dead embryos), earthworm, ground wood pulp, margarine, and soil.

FUSARIUM GRAMINEARUM Schwabe

Wollenweber and Reinking, Die Fusarien, pp. 82–83. 1935.

HABITATS: *Aleurites* sp., *Chrysanthemum cinerariaefolium* Vis., *Coffea arabica* L., *Coffea* sp., *Crotalaria juncea* L., Gramineae, *Hordeum vulgare* L., **Triticum aestivum* L., and **Zea mays* L.

FUSARIUM HETEROSPORUM Nees emend. Raillo

Wollenweber and Reinking, Die Fusarien, pp. 72–73. 1935.

Raillo, Fungi of the genus *Fusarium*, pp. 216–217. 1950.

Bilai, The Fusaria, pp. 264–265. 1955.

HABITATS: *Cenchrus ciliatus* L., *Gossypium* sp., *Panicum maximum* Jacq., *Pennisetum glabrum* Steud., *Pennisetum trachyphyllum* Pilger, *Rubus idaeus* L., and *Sorghum versicolor* Anderss.

FUSARIUM SAMBUCINUM Fuckel

Wollenweber and Reinking, Die Fusarien, pp. 75–76. 1935.

HABITATS: *Cucurbita* sp., *Lactuca sativa* L. *Lycopersicum esculentum* Mill., **Melinis minutiflora* Beauv., *Picea sitchensis* Carr., *Solanum tuberosum* L., *Triticum aestivum* L., air sac of a turkey, and soil.

FUSARIUM SAMBUCINUM var. COERULEUM Wr.

Wollenweber, Ann. Mycol. 15: 55. 1917.

Gordon, Can. J. Botany, 30: 227 and 230. 1952.

Syn. *F. sambucinum* Fuckel forma 1 Wr.

Wollenweber and Reinking, Die Fusarien, pp. 76–77. 1935.

HABITATS: *Dahlia* sp., *Lactuca sativa* L., *Picea sitchensis* Carr., *Rubus idaeus* L., *Solanum tuberosum* L., and soil.

FUSARIUM SAMBUCINUM Fuckel forma 6 Wr.

Wollenweber and Reinking, Die Fusarien, p. 78. 1935.

Gordon, Can. J. Botany, 30: 230–231. 1952.

HABITATS: *Heracleum sphondylium* L., *Humulus lupulus* L., *Polygonum sieboldii* de Vriese, *Solanum tuberosum* L., *Triticum aestivum* L., *Urtica dioica* L., and *Viola* sp.

FUSARIUM TUMIDUM Sherb.

Wollenweber and Reinking, Die Fusarien, pp. 83–84. 1935.

HABITAT: *Coffea* sp.

Section Lateritium Wr.

Wollenweber and Reinking, Die Fusarien, pp. 86–88. 1935.

This section of the genus was represented among the various isolates of *Fusarium* by *F. lateritium* Nees, *F. lateritium* forma *cajanae* (Padw.) Gordon, *F. lateritum* forma *crotalariae* (Padw.) Gordon, and *F. stilboides* Wr.

FUSARIUM LATERITIUM Nees

Wollenweber and Reinking, Die Fusarien, pp. 88–90. 1935.

HABITATS: *Albizzia* sp., *Aleurites* sp., *Citrus limonia* Osbeck, *Citrus* sp., *Chrysanthemum cinerariaefolium* Vis., *Corylus* sp., *Euonymus europaeus* L., *Fraxinus excelsior* L., *Lupinus* sp., *Malus sylvestris* Mill., *Prunus* sp., *Ribes* sp., *Rubus idaeus* L., and *Ulmus* sp.

FUSARIUM LATERITIUM Nees forma CAJANAE (Padw.) Gordon
Gordon, Can. J. Botany, 30: 232. 1952.
Syn. *F. udum* Butl. var. *cajani* Padw.
Padwick, Indian J. Agr. Science, 10: 877. 1940.
HABITAT: *Cajanus indicus* L.

FUSARIUM LATERITIUM Nees forma CROTALARIAE (Padw.) Gordon
Gordon, Can. J. Botany, 30: 232–233. 1952.
Syn. *F. udum* Butl. var. *crotalariae* Padw.
Padwick, Indian J. Agr. Science, 10: 877. 1940.
HABITAT: *Crotalaria juncea* L.

FUSARIUM STILBOIDES Wr.
Wollenweber and Reinking, Die Fusarien, pp. 94–95. 1935.
HABITATS: *Citrus* sp., *Coffea arabica* L., *Coffea robusta* L. Linden, *Coffea* sp., *Lupinus* sp., and *Prunus persica* Batsch.

Section Liseola Wr., Sherb., Rg., Johann, & Bailey

Wollenweber, Sherbakoff, Reinking, Johann, and Bailey, J. Agr. Research, 30: 841. 1925.

Wollenweber and Reinking, Die Fusarien, pp. 96–98. 1935.

This section of the genus was represented among the various isolates of *Fusarium* by *F. moniliforme* Sheld., *F. moniliforme* var. *anthophilum* (A. Br.) Wr., and *F. moniliforme* var. *subglutinans* Wr. & Rg.

FUSARIUM MONILIFORME Sheld.
Wollenweber and Reinking, Die Fusarien, pp. 98–99. 1935.
HABITATS: *Arachis hypogaea* L., *Betula* sp., *Citrullus vulgaris* Schrad., *Coffea arabica* L., *Crotalaria juncea* L., *Dianthus caryophyllus* L., *Elaeis guineensis* Jacq., *Gossypium* sp., Gramineae, **Ipomoea pes-caprae* Sweet, *Linum usitatissimum* L., *Lycopersicum esculentum* Mill., *Musa paradisiaca* L. var. *sapientum* Kuntze, *Oryza sativa* L., *Pennisetum typhoideum* L. Rich., *Ricinus communis* L., *Saccharum officinarum* L., *Sorghum vulgare* L., *Sorghum* sp., *Triticum aestivum* L., **Zea mays* L., sewage treatment plant, soil, and water.

FUSARIUM MONILIFORME Sheld. var. ANTHOPHILUM (A. Br.) Wr.
Wollenweber and Reinking, pp. 101–102. 1935.
HABITAT: *Solanum melongena* L. var. *esculentum* Nees.

FUSARIUM MONILIFORME Sheld. var. SUBGLUTINANS Wr. & Rg.
Wollenweber and Reinking, Die Fusarien, p. 100. 1935.
HABITATS: *Ananas sativus* Schult., *Hordeum brachyantherum* Nevski, *Saccharum officinarum* L., *Triticum aestivum* L., and **Zea mays* L.

Section Elegans Wr.

Wollenweber and Reinking, Die Fusarien, pp. 104–109. 1935.

Gordon, Can. J. Botany, 30: 236. 1952.

This section of the genus was represented by *Fusarium oxysporum* Schl. emend. S. & H. and *F. oxysporum* var. *redolens* (Wr.) Gordon. Fourteen pathogenic formae of *F. oxysporum* were distinguished among the various isolates.

FUSARIUM OXYSPORUM Schl. emend. S. & H. pr.p.
Wollenweber and Reinking, Die Fusarien, pp. 117–118. 1935.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.
HABITATS: *Agave sisalana* (Engelm.) Perrine, *Albizzia julibrissin* Durazz., *Amomum subulatum* Roxb., *Ammophila arenaria* Host., *Arachis hypogaea* L., *Asparagus plumosus* Baker, *Beta vulgaris* L., *Betula* sp., *Capsicum annum* L., *Citrullus vulgaris* Schrad., *Citrus maxima*

Merr., *Citrus sinensis* Osbeck, *Citrus* sp., *Clarkia elegans* Pursh., *Corchorus olitorius* L., *Crotalaria anagyroides* H. B. K., *Cucumis sativus* L., *Coffea arabica* L., *Coffea robusta* L. Linden, *Coffea* sp., *Daucus carota* L. var. *sativa* DC., *Dianthus caryophyllus* L., *Elaeis guineensis* Jacq., *Gossypium* sp., *Helianthus annuus* L., *Humulus lupulus* L., *Iris* sp., *Lactuca sativa* L., *Lilium candidum* L., *Linum usitatissimum* L., *Lupinus luteus* L., *Lupinus* sp., *Lycopersicum esculentum* Mill., *Medicago sativa* L., *Musa paradisiaca* L. var. *sapientum* Kuntze, *Narcissus pseudo-narcissus* L., *Odontoglossum* sp., *Panicum maximum* Jacq., *Passiflora edulis* Sims., *Passiflora* sp., *Pelargonium* sp., *Persea gratissima* Gaertn., *Picea morinda* Link, *Piper betle* L., *Piper nigrum* L., *Pisum sativum* L., *Prunus communis* Arcang., *Ricinus communis* L., *Saccharum officinarum* L., *Sesamum indicum* L., *Solanum melongena* L. var. *esculentum* Nees, *Solanum tuberosum* L., *Thea sinensis* L., *Theobroma cacao* L., *Trifolium alexandrinum* L., *Trifolium pratense* L., *Triticum aestivum* L., *Vicia faba* L., *Zea mays* L., cellophas-based fountain solution, dung, goose eggs (shell membranes with dead embryos), sewage treatment plant, soil, and zinc sulphate solution (16.1%).

FUSARIUM OXYSPORUM Schl. var. REDOLENS (Wr.) Gordon

Gordon, Can. J. Botany, 30: 236 and 238. 1952.

Syn. *F. redolens* Wr.

Wollenweber and Reinking, Die Fusarien, pp. 126–127. 1935.

Section Elegans Wr. subsection Pseudomartiella Raillo

Raillo, Fungi of the genus *Fusarium*, p. 257. 1950.

F. solani (Mart.) App. & Wr. var. *redolens* (Wr.) Bilai

Bilai, The Fusaria, p. 288. 1955.

HABITATS: *Arachis hypogaea* L., *Picea sitchensis* Carr., *Pisum sativum* L., *Prunus cerasifera* Ehrh., *Pyrus communis* L., *Sorghum* sp., *Vicia faba* L., in living human tissue beneath fingernail, and soil.

FUSARIUM OXYSPORUM Schl. forma CALLISTEPHI (Beach) S. & H.

Snyder and Hansen, Am. J. Botany, 27: 66. 1940.

Syn. *F. conglutinans* Wr. var. *callistephi* Beach

Beach, Mich. Acad. Sci. Report, 20: 281–308. 1918.

HABITAT: *Callistephus chinensis* Nees (China aster).

FUSARIUM OXYSPORUM Schl. forma CEPAE (Hanz.) S. & H.

Snyder and Hansen, Am. J. Botany, 27: 66. 1940.

Syn. *F. cepae* Hanz.

Hanzawa, Mycol. Centralblatt, 5: 4–13. 1914.

HABITAT: *Allium cepa* L. (onion).

FUSARIUM OXYSPORUM Schl. forma CUBENSE (E.F.S.) S. & H.

Snyder and Hansen, Am. J. Botany, 27: 66. 1940.

Syn. *F. oxysporum* Schl. var. *cubense* (E.F.S.) Wr. & Rg.

Wollenweber and Reinking, Die Fusarien, pp. 119–120. 1935.

HABITAT: *Musa paradisiaca* L. var. *sapientum* Kuntze (banana).

FUSARIUM OXYSPORUM Schl. forma DIANTHI (Prill. & Del.) S. & H.

Snyder and Hansen, Am. J. Botany, 27: 66. 1940.

Syn. *F. dianthi* Prill & Del.

Delacroix, Ann. Nat. Agron., 16: 161–201. 1900.

HABITAT: *Dianthus caryophyllus* L. (carnation).

FUSARIUM OXYSPORUM Schl. forma GLADIOLI (Massey) S. & H.

Snyder and Hansen, Am. J. Botany, 27: 66. 1940.

Buxton, Trans. Brit. Mycol. Society, 38: 193–201. 1955.

Syn. *F. oxysporum* Schl. var. *gladioli* Massey

Massey, Phytopathology, 16: 509–523. 1926.

F. orthoceras Wr. var. *gladioli* McCull.

McCulloch, Phytopathology, 34: 263–287. 1944.

HABITAT: *Gladiolus* spp. (gladiolus).

FUSARIUM OXYSPORUM Schl. forma LINI (Bolley) S. & H.

Snyder and Hansen, Am. J. Botany, 27: 66. 1940.

Syn. *F. lini* Bolley

Wollenweber and Reinking, Die Fusarien, p. 113. 1935.

HABITAT: *Linum usitatissimum* L. (flax).

FUSARIUM OXYSPORUM Schl. forma LYCOPERSICI (Sacc.) S. & H.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.
Syn. *F. bulbigenum* Cke. & Mass. var. *lycopersici* (Brushi) Wr. & Rg.
Wollenweber and Reinking, Die Fusarien, pp. 114–115. 1935.
HABITAT: *Lycopersicum esculentum* Mill. (tomato).

FUSARIUM OXYSPORUM Schl. forma NICOTIANAE (Johns.) S. & H.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.
Syn. *F. oxysporum* Schl. var. *nicotianae* Johns.
Wollenweber and Reinking, Die Fusarien, p. 120. 1935.
HABITAT: *Nicotiana tabacum* L. (tobacco).

FUSARIUM OXYSPORUM Schl. forma NIVEUM (E.F.S.) S. & H.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.
Syn. *F. bulbigenum* Cke. & Mass. var. *niveum* (E.F.S.) Wr.
Wollenweber and Reinking, Die Fusarien, p. 117. 1935.
HABITAT: *Citrullus vulgaris* Schrad. (watermelon).

FUSARIUM OXYSPORUM Schl. forma PASSIFLORAE Purss
Purss, Queensland J. Agr. Science, 11: 79–81. 1954.
HABITAT: *Passiflora edulis* Sims. (purple granadilla).

FUSARIUM OXYSPORUM Schl. forma PINI (Hartig) S. & H.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.
Syn. *F. bulbigenum* Cke. & Mass. var. *blasticola* (Rostrup) Wr.
Wollenweber and Reinking, Die Fusarien, p. 115. 1935.
HABITATS: *Larix sibirica* Ledeb. (siberian larch), *Picea abies* Karst. (Norway spruce), and *Pinus sylvestris* L. (Scots pine).

FUSARIUM OXYSPORUM Schl. forma PISI (Lindf.) S. & H.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.
Syn. *F. orthoceras* App. & var. *pisi* Lindf.
Wollenweber and Reinking, Die Fusarien, p. 112. 1935.
HABITAT: *Pisum sativum* L. (pea).

FUSARIUM OXYSPORUM Schl. forma TUBEROSI S. & H.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.
Syn. *F. oxysporum* Schl. form 1 Wr.
Wollenweber and Reinking, Die Fusarien, p. 119. 1935.
HABITAT: *Solanum tuberosum* L. (potato).

FUSARIUM OXYSPORUM Schl. forma VASINFECTUM (Atk.) S. & H.
Snyder and Hansen, Am. J. Botany, 27: 66. 1940.
Syn. *F. vasinfectum* Atk.
Wollenweber and Reinking, Die Fusarien, p. 124. 1935.
HABITAT: *Gossypium* spp. (cotton).

Section Martiella Wr.

Wollenweber and Reinking, Die Fusarien, pp. 127–129. 1935.
Snyder and Hansen, Am. J. Botany, 28: 738–740. 1941.
Gordon, Can. J. Botany, 30: 238–239. 1952.

Two species of *Fusarium*, namely *F. coeruleum* (Lib.) Sacc. and *F. solani* (Mart.) App. & Wr. emend. S. & H., belonging in this section of the genus, were encountered among the various isolates.

FUSARIUM COERULEUM (Lib.) Sacc.
Wollenweber and Reinking, Die Fusarien, p. 134. 1935.
Gordon, Can. J. Botany, 30: 245. 1952.
HABITAT: *Solanum tuberosum* L.

FUSARIUM SOLANI (Mart.) App. & Wr. emend. S. & H.
Snyder and Hansen, Am. J. Botany, 28: 740. 1941.
Snyder and Hansen, Am. J. Botany, 32: 662. 1945.
Gordon, Can. J. Botany, 30: 239–240. 1952.

HABITATS: *Agave sisalana* (Engelm.) Perrine, *Albizzia julibrissin* Durazz., *Albizzia procera* Benth., *Allium cepa* L., *Antirrhinum majus* L., *Arachis hypogaea* L., *Beta vulgaris* L., *Boehmeria nivea* Gaud., *Callistephus chinensis* Nees, *Carica papaya* L., *Capsicum annum* L., *Cephaelis ipecacuanha* Rich., *Ceratonia siliqua* L., *Cicer arietinum* L., *Citrullus vulgaris* Schrad., *Citrus aurantifolia* Swingle, *Citrus aurantium* L., *Citrus limonia* Osbeck, *Citrus maxima* Merr., *Citrus sinensis* Osbeck, *Citrus* sp., *Cocos nucifera* L., *Coffea arabica* L., *Coffea robusta* L. Linden, *Coffea* sp., *Corchorus olitorius* L., *Crotalaria anagyroides* H.B.K., *Dahlia* sp., *Daucus carota* L. var. *sativa* DC., *Digitalis purpurea* L., *Elaeis guineensis* Jacq., *Enterolobium saman* Prain, *Gladiolus* sp., *Gossypium* sp., *Helianthus annuus* L., *Hevea* sp., *Hibiscus esculentus* L., *Hyacinthus* sp., *Hyoscyamus niger* L., *Ipomoea pes-caprae* Sweet, *Lachenalia glaucina* Jacq., *Lactuca sativa* L., *Linum usitatissimum* L., *Lupinus luteus* L., *Lycopersicum esculentum* Mill., *Malus sylvestris* Mill., *Mangifera indica* L., *Medicago sativa* L., *Melilotus alba* Desr., *Morus nigra* L., *Musa paradisiaca* L. var. *sapientum* Kuntze, *Pisum sativum* L., *Piper nigrum* L., *Pittosporum tobira* Ait., *Poinciana regia* Bojer, *Prunus communis* Arcang., *Psidium gujava* L., *Sesbania* sp., *Solanum melongena* L. var. *esculentum* Nees, *Solanum tuberosum* L., *Theobroma cacao* L., *Thunia* sp., *Trifolium alexandrinum* L., *Trifolium pratense* L., *Tropaeolum majus* L., *Vicia faba* L., *Vigna sinensis* Savi, *Vitis* sp., air, beetle, capsid, cooling tower slats (*Sequoia* sp.), cotton filters, contaminant in a culture of *Thamnidium elegans*, dung, sewage treatment plant, and soil.

Distribution of Species by Habitats

PLANTS

Agave sisalana (Engelm.) Perrine (sisal hemp)
F. oxysporum (Kenya–Uganda)
F. solani (Tanganyika)

Agrostis sp. (bent grass)
F. nivale (United States)

Albizzia julibrissin Durazz
F. oxysporum, F. solani (Iran)

Albizzia procera Benth.
F. solani (India)

Albizzia sp.
F. lateritium (Kenya–Uganda)

Aleurites montana Wils.
F. arthrosporioides (Malaya)

Aleurites sp.
F. graminearum, F. lateritium (Nyasaland)

Allium cepa L. (onion)
F. oxysporum f. *cepae* (Australia)
F. solani (Ceylon)

Ammophila arenaria Host. (European beach-grass)
F. oxysporum (England)

Amomum subulatum Roxb. (Greater cardamom)
F. oxysporum (India)

Anacardium occidentale L. (cashew)
F. decemcellulare (Ghana)

Ananas sativus Schult. (pineapple)
F. moniliforme var. *subglutinans* (South Africa)
F. solani (Kenya–Uganda)

Antirrhinum majus L. (snapdragon)
F. equiseti, F. solani (N. Rhodesia)

Arachis hypogaea L. (peanut)
F. equiseti (India; Tanganyika)
F. compactum (Tanganyika)
F. semitectum, F. moniliforme, F. oxysporum var. *redolens* (India)
F. oxysporum (India; N. Rhodesia; Tanganyika)

Asparagus plumosus Baker (asparagus fern)
F. oxysporum (Guernsey)

Avena sativa L. (oat)
F. avenaceum (Scotland; United States)
F. nivale (Scotland)

Beta vulgaris L. (beet)
F. acuminatum (Scotland)
F. oxysporum, F. solani (Turkey)

Betula sp. (birch)
F. moniliforme, F. oxysporum (Finland)

Boehmeria nivea Gaud. (ramie)
F. solani (British Honduras)

Bombax malabaricum DC. (red cotton tree)
F. semitectum (India)

Bothriochloa insculpta (Hochst.) A. Camus
F. equiseti (Kenya–Uganda)

Bromus marginatus Nees
F. avenaceum (Kenya–Uganda)

Cajanus indicus Spreng. (pigeon pea)
F. lateritium f. *cajani* (Tanganyika)

Callistephus chinensis Nees (China aster)
F. equiseti, F. oxysporum f. *callistephi* (Scotland)
F. solani (S. Rhodesia)

Capsicum annuum L. (red pepper)
F. semitectum (Kenya–Uganda)
F. oxysporum (Greece)
F. solani (Greece; Tanganyika)

Carica papaya L. (papaya)
F. equiseti (N. Rhodesia; Tanganyika)
F. solani (Malaya; N. Rhodesia; Tanganyika)

Cenchrus ciliaris L.
F. heterosporum (Kenya–Uganda)

Cephaelis ipecacuanha Rich. (ipecacuanha)
F. solani (India)

Ceratonia siliqua L. (carob)
F. solani (Israel)

Chenopodium album L. (lamb's-quarters)
F. avenaceum (Scotland)

Chloris gayana Kunth. (Rhodes grass)
F. equiseti (Kenya–Uganda)
Chrysanthemum cinerariaefolium Vis. (pyrethrum)
F. semitectum, F. equiseti, F. graminearum, F. lateritium (Kenya–Uganda)
Cicer arietinum L. (chick pea)
F. solani (Egypt)
Citrullus vulgaris Schrad. (watermelon)
F. moniliforme, F. oxysporum f. *niveum, F. solani* (Egypt)
F. oxysporum (Israel)
Citrus aurantifolia Swingle (lime)
F. semitectum, F. equiseti (Israel)
F. solani (Israel; Tanganyika)
Citrus aurantium L. (sour orange)
F. solani (India)
Citrus limonia Osbeck (lemon)
F. equiseti, F. lateritium, F. solani (Israel)
Citrus maxima Merr. (grapefruit)
F. oxysporum (Israel)
F. solani (Israel; Tanganyika)
Citrus medica L. (citron)
F. semitectum (Israel)
Citrus sinensis Osbeck (Valencia orange)
F. oxysporum, F. solani (Israel)
Citrus spp.
F. poae, F. semitectum, F. moniliforme, F. oxysporum, F. solani (Israel)
F. oxysporum, F. stilboides (South Africa)
F. equiseti, F. lateritium (Kenya–Uganda)
F. moniliforme, F. solani (S. Rhodesia)
Clarkia elegans Pursh. (clarkia)
F. oxysporum (England)
Cocos nucifera L. (coconut)
F. solani (Ghana)
Coffea arabica L. (arabian coffee)
F. graminearum, F. lateritium, F. moniliforme (Nyasaland)
F. solani (Tanganyika)
Coffea robusta L. Linden
F. oxysporum (Belgian Congo)
Coffea arabica L. and *C. robusta* L. Linden
F. decemcellulare, F. semitectum, F. tumidum, F. stilboides, F. oxysporum, F. solani (Portugal)
Coffea spp.
F. equiseti, F. solani (Kenya–Uganda)
F. graminearum (Nyasaland)
F. lateritium (Belgian Congo; Tanganyika)
F. stilboides (Kenya–Uganda; Nyasaland; S. Rhodesia; Tanganyika)
F. oxysporum, F. solani (Tanganyika)
Corchorus olitorius L. (jute)
F. oxysporum (India)
F. solani (Pakistan)
Coriandrum sativum L. (coriander)
F. oxysporum (India)
Corylus sp. (hazel nut)
F. lateritium (England)
Crotalaria anagyroides H.B.K.
F. oxysporum, F. solani (New Guinea)
Crotalaria juncea L. (sunn hemp)
F. acuminatum, F. lateritium f. *crotalariae* (S. Rhodesia)
F. equiseti (Nyasaland; S. Rhodesia)
F. moniliforme (Kenya–Uganda)
Crotalaria sp.
F. graminearum (Kenya–Uganda)
Cucumis sativus L. (cucumber)
F. oxysporum (Kenya–Uganda)
Cucurbita pepo L. (vegetable marrow (*a*); pumpkin (*b*))
F. culmorum (Malta (*a*))
F. equiseti (Malta (*a*); S. Rhodesia (*b*))
Cucurbita sp.
F. sambucinum (England)
Cuminum cyminum L. (cumin)
F. oxysporum (India)
Cynodon dactylon Pers. (Bahama-grass)
F. equiseti (Kenya–Uganda; United States)
F. sporotrichioides (United States)
Cynodon plectostachyum Pilger
F. equiseti (Kenya–Uganda)
Cytisus scoparius Link (broom)
F. avenaceum, F. equiseti (Kenya–Uganda)
Dactylis glomerata L. (orchard grass)
F. avenaceum (Kenya–Uganda)
Dahlia sp. (dahlia)
F. equiseti, F. solani (Israel)
F. sambucinum var. *coeruleum* (Scotland)
Daucus carota L. var. *sativa* DC. (carrot)
F. oxysporum, F. solani (Malaya)
Dianthus caryophyllus L. (carnation)
F. culmorum (Denmark)
F. acuminatum, F. culmorum (Scotland)
F. equiseti, F. moniliforme (S. Rhodesia)
F. avenaceum (Denmark; Scotland; S. Rhodesia)
F. oxysporum (Israel; Kenya–Uganda)
F. oxysporum f. *dianthi* (Denmark; England; Scotland; N. Rhodesia)
Digitalis purpurea L. (foxglove)
F. solani (India)
Durio zibethinus Murr. (durian)
F. decemcellulare (Malaya)
Eclipta alba (L.) Hassk.
F. semitectum (India)
Elaeis guineensis Jacq. (oil palm)
F. dimerum, F. oxysporum (Belgian Congo)
F. oxysporum, F. solani (Belgian Congo; Nigeria)
F. moniliforme (Nigeria)
Enterolobium saman Prain (rain tree)
F. solani (India)
Eucalyptus sp. (gum)
F. equiseti (Sudan)
Euonymus europaeus L. (European spindle tree)
F. lateritium (Isle of Rhum, Hebrides)
Fraxinus excelsior L. (ash)
F. lateritium (England)
Gladiolus spp. (gladiolus)
F. culmorum (Israel)
F. oxysporum f. *gladioli* (Australia; England; Kenya–Uganda; S. Rhodesia)
F. solani (India; S. Rhodesia)
Gossypium spp. (cotton)
F. semitectum (Aden)
F. culmorum, F. moniliforme (Egypt)
F. oxysporum, F. solani (Egypt; Sudan)

Gossypium spp. (cotton)—*Cont'd.*
F. oxysporum f. *vasinfectum* (Egypt; Tanganyika)
F. equiseti (Egypt; S. Rhodesia; Sudan)
F. heterosporum (Sudan)
Gramineae
F. avenaceum (Venezuela; Wales)
F. culmorum (Wales)
F. graminearum, F. moniliforme (Honduras)
F. nivale F. avenaceum, F. culmorum (New Zealand)
Helianthus annuus L. (sunflower)
F. solani (Uruguay)
F. equiseti (Uruguay; Chile)
F. oxysporum (Nyasaland; Uruguay)
Heracleum sphondylium L. (cow parsnip)
F. avenaceum, F. sambucinum f. 6 (England)
Hevea sp.
F. equiseti (Ghana)
F. solani (Malaya)
Hibiscus esculentus L. (okra)
F. solani (India)
Hibiscus cannabinus L.
F. semitectum (Kenya–Uganda)
Hordeum brachyantherum Nevski (meadow barley)
F. moniliforme var. *subglutinans* (United States)
Hordeum vulgare L. (barley)
F. sporotrichioides (England)
F. nivale (Scotland)
F. equiseti (Kenya–Uganda)
F. culmorum (Sweden)
F. graminearum (United States)
Humulus lupulus L. (hop)
F. sambucinum f. 6, *F. oxysporum* (Tasmania)
Hyacinthus sp.
F. solani (South Africa)
Hyoscyamus niger L. (henbane)
F. solani (India)
Ipomoea pes-caprae Sweet
F. moniliforme, F. solani (Ceylon)
Iris sp.
F. oxysporum (Guernsey)
Lachenalia glaucina Jacq.
F. solani (Scotland)
Lactuca sativa L. (lettuce)
F. equiseti, F. culmorum, F. sambucinum, F. s. var. *coeruleum, F. oxysporum, F. solani* (England)
Larix sibirica Ledeb. (siberian larch)
F. oxysporum f. *pini* (Finland)
Lathyrus odoratus L. (sweet pea)
F. avenaceum (Scotland)
Lilium candidum L. (madonna lily)
F. oxysporum (Mauritius)
Linum usitatissimum L. (flax)
F. acuminatum, F. oxysporum (Australia)
F. culmorum (Wales)
F. moniliforme (Portugal)
F. avenaceum (Kenya–Uganda; Portugal)
F. equiseti (Australia; Kenya–Uganda)
F. oxysporum f. *lini* (Australia; New Zealand: Portugal; Wales)
Lolium perenne L. (rye grass)
F. acuminatum (Scotland)
F. equiseti, F. culmorum (Wales)
Lotus corniculatus L. (trefoil)
F. avenaceum (Scotland)
Lupinus luteus L. (yellow lupine)
F. oxysporum, F. solani (Tanganyika)
Lupinus spp.
F. lateritium, F. oxysporum (Kenya–Uganda)
F. lateritium (Tanganyika)
Lycopersicum esculentum Mill. (tomato)
F. acuminatum (Israel; Turkey)
F. compactum (Australia)
F. solani (Greece)
F. semitectum, F. moniliforme (Egypt)
F. sambucinum (Egypt; Kenya–Uganda)
F. equiseti (Egypt; Ghana; Israel; Malta; Turkey)
F. oxysporum (Egypt; Scotland; Greece; Israel)
F. oxysporum f. *lycopersici* (Egypt; Israel; S. Rhodesia)
Malus sylvestris Mill. (apple)
F. avenaceum (Scotland)
F. acuminatum (United States)
F. lateritium (S. Rhodesia)
F. dimerum, F. equiseti (Israel)
F. oxysporum (India)
F. solani (India; Israel)
Mangifera indica L. (mango)
F. solani (Nigeria)
Manihot utilissima Pohl (cassava)
F. equiseti (Nigeria)
Medicago sativa L. (alfalfa)
F. avenaceum (Scotland)
F. acuminatum, F. equiseti, F. culmorum, F. oxysporum, F. solani (Mexico)
Melilotus alba Desr. (white sweet clover)
F. solani (United States)
Melinis minutiflora Beauv. (molasses grass)
F. sambucinum (Kenya–Uganda)
Morus nigra L. (black mulberry)
F. culmorum, F. solani (England)
Muhlenbergia racemosa (Michx.) B.S.P.
F. avenaceum (United States)
Musa paradisiaca L. var. *sapientum* Kuntze
F. dimerum, F. moniliforme (Jamaica)
F. compactum (Sudan)
F. semitectum (Kenya–Uganda; Sudan)
F. equiseti (Egypt; Israel; Jamaica; Kenya–Uganda; Sudan)
F. oxysporum (England; Honduras; Israel; Jamaica; Kenya–Uganda; Tanganyika)
F. oxysporum f. *cubense* (British Cameroons; French West Africa; Ghana; Kenya–Uganda; Malaya; Mauritius; Sarawak; Tanganyika)
F. solani (Honduras; Israel; Jamaica; Sudan)
Narcissus pseudo-narcissus L. (narcissus)
F. oxysporum (South Africa)
Nicotiana tabacum L. (tobacco)
F. moniliforme (S. Rhodesia)
F. oxysporum f. *nicotianae* (Nyasaland)
Odontoglossum sp. (orchid)
F. oxysporum (England)

Ornithopus sativus Brot.
F. equiseti (Kenya–Uganda)
Oryza sativa L. (rice)
F. equiseti (India)
F. moniliforme (Sierra Leone)
Panicum maximum Jacq. (Guinea-grass)
F. heterosporum, F. oxysporum (Kenya–Uganda)
Paspalum dilatatum Poir (dallis-grass)
F. avenaceum (Australia)
Passiflora edulis Sims. (purple granadilla)
F. equiseti, F. oxysporum, F. solani (Kenya–Uganda)
F. oxysporum (Nyasaland)
F. oxysporum f. *passiflorae* (Australia)
Pelargonium sp.
F. oxysporum (Kenya–Uganda)
Persea gratissima Gaertn. (avocado)
F. oxysporum (Tanganyika)
Pennisetum mairva Staff. & Hubbard
F. equiseti (Gambia)
Pennisetum glabrum Steud.
F. heterosporum (Kenya–Uganda)
Pennisetum trachyphyllum Pilg.
F. heterosporum (Kenya–Uganda)
Pennisetum typhoideum L. Rich.
F. moniliforme (N. Nigeria)
Petroselinum sativum Hoffm. (parsley)
F. solani (Kenya–Uganda)
Phaseolus vulgaris L. (bean)
F. equiseti (N. Rhodesia)
Picea abies Karst (Norway spruce)
F. oxysporum f. *pini* (Finland)
Picea morinda Link
F. acuminatum, F. oxysporum (India)
Picea sitchensis Car. (sitka spruce)
F. sambucinum, F. s. var. *coeruleum, F. oxysporum* var. *redolens* (England)
Pinus caribaea Morelet. (slash pine)
F. acuminatum, F. equiseti (Australia)
Pinus elliotti Engelm.
F. compactum (Australia)
Pinus sylvestris L. (Scots pine)
F. oxysporum f. *pini* (Finland)
Pinus sp.
F. avenaceum (England)
Piper betle L. (betel leaf)
F. semitectum (India)
F. oxysporum (Egypt)
Piper nigrum L. (pepper)
F. decemcellulare, F. oxysporum, F. solani (Sarawak)
Piper sp.
F. equiseti, F. oxysporum (Turkey)
Pisum sativum L. (pea)
F. avenaceum, F. oxysporum var. *redolens, F. solani* (England)
F. oxysporum (England; Sudan)
F. oxysporum f. *pisi* (Israel; New Zealand; S. Rhodesia)
Pittosporum tobira Ait. (Japanese pittosporum)
F. solani (Israel)
Poiciana regia Bojer (flamboyant)
F. equiseti (Israel)
F. solani (S. Rhodesia)
Polygonum sieboldii de Vriese (Japanese knotweed)
F. sambucinum f. 6 (England)
Prunus armeniaca L. (apricot)
F. equiseti (Turkey)
Prunus cerasifera Ehrh. (myrobalan plum)
F. oxysporum var. *redolens* (Israel)
Prunus communis Arcang. (almond)
F. oxysporum, F. solani (Israel)
Prunus persica Batsch (peach)
F. stilboides (Australia)
Prunus sp.
F. lateritium (S. Rhodesia)
Psidium guajava L. (guava)
F. solani (India)
Pyrus communis L. (pear)
F. merismoides, F. oxysporum var. *redolens* (United States)
Quercus sp. (oak)
F. oxysporum (India)
Ribes sp. (gooseberry)
F. lateritium (Isle of Unst, Shetlands)
Ricinus communis L. (castor bean)
F. semitectum, F. oxysporum (Israel)
F. moniliforme (India)
Rubus idaeus L. (red raspberry)
F. culmorum (England)
F. avenaceum, F. heterosporum, F. sambucinum var. *coeruleum, F. lateritium* (Scotland)
Saccharum officinarum L. (sugar cane)
F. moniliforme (Kenya–Uganda; Tanganyika)
F. moniliforme var. *subglutinans* (India; Mauritius)
F. oxysporum (Uruguay)
Schoenus nigricans L. (moorland sedge)
F. acuminatum (Isle of Rhum, Hebrides)
Scirpus americanus Pers.
F. sporotrichioides (England)
Sesamum indicum L. (sesame)
F. oxysporum (Nyasaland)
Sesbania sp. (dhaincha)
F. solani (India)
Solanum melongena L. var. *esculentum* Nees (eggplant)
F. moniliforme var. *anthophilum* (Kenya–Uganda)
F. oxysporum (Egypt; Greece)
F. solani (Greece; India)
Solanum tuberosum L. (potato)
F. acuminatum, F. coeruleum, F. sambucinum f. 6 (Australia)
F. semitectum (Israel)
F. sambucinum (Northern Ireland)
F. culmorum (Scotland)
F. sambucinum var. *coeruleum* (Northern Ireland; Scotland)
F. equiseti (India; Israel)
F. avenaceum (England; Northern Ireland; Scotland)
F. oxysporum (Argentina; Australia; Egypt; India; Israel; Kenya–Uganda; S. Rhodesia; Sudan; United States)
F. solani (India; Israel; Malaya; St. Helena; United States)
Sorghum veriscolor Anderss.
F. heterosporum (Nyasaland)

Sorghum vulgare Pers. (sorghum)
F. equiseti (N. Nigeria)
F. moniliforme (Pakistan)
Sorghum sp.
F. semitectum (Tanganyika)
F. equiseti (Thailand)
F. moniliforme, *F. oxysporum* var. *redolens* (Israel)
Thea sinensis L. (tea)
F. oxysporum (Kenya–Uganda)
Theobroma cacao L. (cocoa)
F. decemcellulare (Ghana; Spanish Guinea)
F. solani (Ghana; Sierra Leone; Nigeria)
F. equiseti, *F. oxysporum* (Ghana; Nigeria)
Thunia sp.
F. solani (England)
Trifolium alexandrinum L. (Egyptian clover)
F. oxysporum, *F. solani* (Israel)
Trifolium pratense L. (red clover)
F. oxysporum, *F. solani* (United States)
Trifolium repens L. (white clover)
F. avenaceum (Scotland)
Triticum aestivum L. (common wheat)
F. decemcellulare, *F. sambucinum* (England)
F. compactum, *F. moniliforme* (India)
F. poae (England; United States)
F. nivale (Netherlands)
F. sambucinum f. 6 (New Zealand)
F. avenaceum, *F. sporotrichioides* (England; New Zealand)
F. equiseti (Australia; New Zealand; United States)
F. graminearum (Argentina; Australia; Kenya–Uganda; New Zealand)
F. culmorum (England; Netherlands; New Zealand)
F. moniliforme var. *subglutinans* Australia)
F. oxysporum (England; Kenya–Uganda; S. Rhodesia)
Tropaeolum majus L. (nasturtium)
F. solani (Egypt)
Ulmus sp. (elm)
F. lateritium (England)
Urtica dioica L. (stinging nettle)
F. sambucinum f. 6 (England)
Vicia faba L. (broad bean)
F. avenaceum, *F. oxysporum*, *F. oxysporum* var. *redolens*, *F. solani* (Cyprus)
F. equiseti (Cyprus; Sudan)
Vicia sativa L. (vetch)
F. avenaceum (Kenya–Uganda)
Vigna sinensis Savi (cowpea)
F. solani (N. Nigeria)
Viola sp. (violet)
F. sambucinum f. 6 (Scotland)
Vitis sp. (grape)
F. acuminatum, *F. solani* (Israel)
Zea mays L. (corn)
F. semitectum, *F. equiseti* (Malaya)
F. poae (Uruguay)
F. culmorum (England)
F. graminearum (N. Rhodesia; S. Rhodesia)
F. moniliforme (Egypt; Ghana; India; Israel; Uruguay; N. Rhodesia; S. Rhodesia)
F. moniliforme var. *subglutinans* (Australia; S. Rhodesia)
F. oxysporum (Kenya–Uganda; S. Rhodesia)

MISCELLANEOUS HABITATS

Air
F. equiseti (Israel)
F. solani (Pakistan)
Chemical solution
F. oxysporum (United States)
Cooling tower slats (*Sequoia* sp.)
F. solani (Australia)
Cotton filters, river water filtering
F. merismoides (England)
Dairy products, utensils, effluent
F. merismoides, *F. avenaceum* (England)
F. dimerum (Kenya–Uganda)
Dung
F. sporotrichioides (England)
F. solani (India)
Earthworm
F. culmorum (England)
Fountain solution, cellophas-based
F. oxysporum (England)
Fowl
F. culmorum, *F. sambucinum*, *F. oxysporum* (England)
Fungi
F. equiseti (Israel)
F. solani (England)
Human tissue
F. oxysporum var. *redolens* (England)
Insects
F. semitectum, *F. solani* (Ghana)
F. solani (South Africa)
Margarine
F. culmorum (England)
Pig
F. sporotrichioides (England)
Sewage, sewage plants, sewage-carrying streams
F. aquaeductuum, *F. merismoides*, *F. dimerum*, *F. poae*, *F. semitectum*, *F. acuminatum*, *F. equiseti*, *F. moniliforme*, *F. oxysporum*, *F. solani* (United States)
Soil
F. dimerum, *F. semitectum* (Pakistan)
F. chlamydosporum (India)
F. avenaceum (Australia; New Zealand; United States)
F. acuminatum (Australia; England)
F. equiseti (Egypt; England; India; Iraq; Nigeria)
F. compactum (Iraq; Sudan)
F. longipes (Sudan)
F. culmorum (England; New Zealand)
F. sambucinum (England; India; Iraq; New Zealand)
F. sambucinum var. *coeruleum* (New Zealand)
F. moniliforme (Egypt; Iraq; Sudan)
F. oxysporum (England; India; Jamaica; New Zealand; United States)

Soil—*Cont'd.*
F. oxysporum var. *redolens* (England)
F. solani (Australia; Ceylon; India; Iraq; Pakistan; Nigeria; Sierra Leone; Sudan; New Zealand)
Stored sugar
F. semitectum (India)
Termite mound
F. equiseti (India)
Water
F. equiseti, *F. moniliforme* (India)
Wood pulp, ground
F. culmorum (England)

Discussion

In "Die Verbreitung der Fusarien in der Natur" published in 1935, Wollenweber and Reinking (15) listed the plants which they had found to be hosts for species of *Fusarium*. They did not indicate the geographic areas from which isolates of *Fusarium* were obtained but it is apparent from the names of the host plants that the various isolates identified by them had originated in tropical and temperate regions. As the various isolates of *Fusarium* that were included in the present study were obtained from tropical and temperate regions from time to time since 1935, the results of this study are complementary to those presented by Wollenweber and Reinking (15). The occurrence of species of *Fusarium* is recorded in the present paper on various species of plants belonging in 32 genera not listed as host plants for these fungi by Wollenweber and Reinking (15). These genera are: *Aleurites*, *Ammophila*, *Amomum*, *Anacardium*, *Boehmeria*, *Bombax*, *Bothriochloa*, *Cenchrus*, *Cephaelis*, *Ceratonia*, *Chloris*, *Clarkia*, *Corchorus*, *Coriandrum*, *Cuminum*, *Digitalis*, *Durio*, *Enterolobium*, *Lachenalia*, *Lactuca*, *Lotus*, *Mangifera*, *Melinis*, *Muhlenbergia*, *Odontoglossum*, *Pittosporum*, *Psidium*, *Schoenus*, *Sesbania*, *Sorghum*, *Thunia*, and *Tropaeolum*. Futhermore, species of plants, classified in 60 genera additional to those just mentioned, are reported to be hosts for certain species of *Fusarium* not reported to occur on them by Wollenweber and Reinking (15).

Of the 26 species of *Fusarium* identified among the various isolates included in this study some were obtained from a greater number of geographic areas and isolated from a wider range of hosts plants than others. Six species were obtained from a relatively large number of geographic areas and from a wide range of host plants. The six species of *Fusarium* found to be most widely distributed are: *F. solani*, obtained from 36 geographic areas, and from 67 species of plants; *F. oxysporum*, from 35 areas and 59 species; *F. equiseti*, from 28 areas and 44 species; *F. moniliforme*, from 22 areas and 21 species; *F. avenaceum*, from 13 areas and 20 species; and *F. semitectum*, from 12 areas and 18 species. On the other hand, five species of *Fusarium*, each represented by only one isolate among approximately 1200 isolates included in this study, must be regarded as being of relatively rare occurrence in nature. These species are: *F. aquaeductuum*, *F. chlamydosporum*, *F. arthrosporioides*, *F. longipes*, and *F. tumidum*.

Species of *Fusarium* were obtained from a wide variety of habitats in addition to plants but only a few species were encountered in each of them with the exception of soil and sewage disposal areas. Thirteen species and two varieties of *Fusarium* were identified among the isolates obtained from soil samples taken from a total of 13 geographic areas some of which were

situated in temperate and others in tropical regions. Ten species were identified among the isolates obtained from sewage, sewage plants, and sewage-carrying streams. Of these 10 species, only one, namely *F. aquaeductuum*, was found in no other habitat. As Peterson and Baker (9) have recently pointed out the fact that *Fusarium* species have seldom been reported to be associated with diseases or injuries of humans, it is worthy of note that one of the isolates of *F. oxysporum* var. *redolens* included in the present study was obtained from living tissue beneath the fingernail of a patient. The usual habitat of this fungus is in soil or in the roots of plants.

References

1. BILAI, B. I. 1955. The Fusaria. Kiev.
2. GORDON, W. L. 1944. The occurrence of *Fusarium* species in Canada. I. Species of *Fusarium* isolated from farm samples of cereal seed in Manitoba. Can. J. Research, C, **22**, 282–286.
3. GORDON, W. L. 1952. The occurrence of *Fusarium* species in Canada. II. Prevalence and taxonomy of *Fusarium* species in cereal seed. Can. J. Botany, **30**, 209–251.
4. GORDON, W. L. 1954. The occurrence of *Fusarium* species in Canada. III. Taxonomy of *Fusarium* species in the seed of vegetables, forage, and miscellaneous crops. Can. J. Botany, **32**, 576–590.
5. GORDON, W. L. 1954. The occurrence of *Fusarium* species in Canada. IV. Taxonomy and prevalence of *Fusarium* species in the soil of cereal plots. Can. J. Botany, **32**, 622–629.
6. GORDON, W. L. 1956. The occurrence of *Fusarium* species in Canada. V. Taxonomy and geographic distribution of *Fusarium* species in soil. Can. J. Botany, **34**, 833–846.
7. GORDON, W. L. 1959. The occurrence of *Fusarium* species in Canada. VI. Taxonomy and geographic distribution of *Fusarium* species on plants, insects, and fungi. Can. J. Botany, **37**, 257–290.
8. GORDON, W. L. 1956. The taxonomy and habitats of the *Fusarium* species in Trinidad, B.W.I. Can. J. Botany, **34**, 847–864.
9. PETERSON, J. E. and BAKER, T. J. 1959. An isolate of *Fusarium roseum* from human burns. Mycologia, **51**, 453–456.
10. RAILLO, A. I. 1950. Grebi roda *Fusarium*. Moscow.
11. SNYDER, W. C. and HANSEN, H. N. 1940. The species concept in *Fusarium*. Am. J. Botany, **27**, 64–67.
12. SNYDER, W. C. and HANSEN, H. N. 1941. The species concept in *Fusarium* with reference to the section Martiella. Am. J. Botany, **28**, 738–742.
13. SNYDER, W. C. and HANSEN, H. N. 1945. The species concept in *Fusarium* with reference to Discolor and other sections. Am. J. Botany, **32**, 657–666.
14. WOLLENWEBER, H. W. and REINKING, O. A. 1935. Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. Paul Parey, Berlin.
15. WOLLENWEBER, E. W. and REINKING, O. A. 1935. Die Verbreitung der Fusarien in der Natur. R. Friedlander und Sohn, Berlin.

MICROFUNGI

V. CONOPLEA PERS. AND EXOSPORIUM LINK[1]

S. J. HUGHES

Abstract

The history of the generic names *Conoplea* Pers. and *Exosporium* Link is traced and many of the early species names are explained from type or authenticated collections. *Conoplea*, typified by *C. sphaerica*, replaces *Streptothrix* Corda. *Streblocaulium* Chev. is probably a synonym of *Conoplea*. Six species and one variety of *Conoplea* are illustrated and described.

Introduction

In this publication, the generic name *Conoplea* Pers. is being used in place of the more recent *Streptothrix* Corda (1839) for six species and one variety. The generic name *Exosporium* Link, which has been in common use for over a hundred years, has recently (Hughes 1958) been put into the synonymy of *Helmisporium* Link. The two names, *Conoplea* and *Exosporium*, are being considered together because, notwithstanding their different structural characteristics, they were brought together as synonyms in 1815 due to a misidentification.

In the preliminary part of this paper the entry of the various pertinent names into mycological history is dealt with in chronological order: in the second part, the six species and one variety of *Conoplea* known to me are illustrated and described.

Historical Part

1797. *Conoplea* was proposed by Persoon with the following generic diagnosis: 'Fila persistentia in formam distinctam compacta, intus pulvere referta'. The name *Conoplea* was derived from '*κόνις*' and '*πλεια*' — abundant spore dust. Persoon included three species, *C. sphaerica*, *C. cylindrica*, and *C. hispidula*.

(1) *Conoplea sphaerica* (Pers.) Pers., Tent. Disp. Meth. Fung., p. 55. 1797.
≡ *Dematium sphaericum* Pers., Usteri's Ann. Bot., 15 Stück: 25. 1795.
≡ *Conoplea olivacea* Fr., Syst. Mycol., 3: 491. 1832/33.

Persoon (1795) described this species as 'gregarium, fasciculis sphaericis subgranulosis pulverulentis nigrescente-olivaceis. . . Provenit ad ramos exsiccatos. . .' The presumed type collection (Fig. 9) bears what has most commonly been referred to as a *Streptothrix*; this species is illustrated (Fig. 3), described, and discussed fully below (pp. 673–679).

(2) *Conoplea cylindrica* Pers., Tent. Disp. Meth. Fung., p. 55. 1797.
≡ *Conoplea comosa* de Brondeau, Mém. Soc. Linn. Paris, 4: 198. 1826.
≡ *Psilonia cylindrica* (Pers.) Fr., Syst. Mycol., 3: 452. 1832/33.
≡ *Chloridium cylindricum* (Pers.) Rabenh., Deutschl. Krypt.-Flora, 1: 75. 1844.

[1]Manuscript received March 21, 1960.
Contribution No. 63 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

Persoon described his species as 'sparsum subcylindricum obscure fuscum. Hab. ad calmum *Arund. Phragm.* [*Phragmites communis*] in sterquiliniis, hyeme, aut tempore vernali; 1½–2 lin. long. lin. 1 crassa; pulvis seminalis copiosus pariter fuscus.' The undoubted type collection (Fig. 10) in Persoon's herbarium in Herb. L[2] (910. 263–437) is labelled '*Conoplea cylindrica* [scr. Pers.] Hb. Pers. [scr. J. H. Schuurmans Stekhoven[3]]'. A preparation from this indicates that the name is based on *Chaetomium elatum* Kunze. It is interesting to note, in this regard, that Sprengel (1827, p. 554) classified Kunze's species in *Conoplea*.

(3) *Conoplea hispidula* Pers., Tent. Disp. Meth. Fung., p. 55. 1797.
≡*Exosporium hispidulum* (Pers.) Nees, Das System. . . . .p. 33. 1816/17.
≡*Dematium hispidulum* (Pers.) Fr., Syst. Mycol., 3: 365. 1832/33.
≡*Sporodum hispidulum* (Pers.) Sacc., Michelia, 2: 25. 1880.
≡*Periconia hispidula* (Pers.) Mason & Ellis, Mycol. Papers, C.M.I., 56: 112. 1953.

Persoon described this as 'sparsa minuta, filis divergentibus rigidis hispida. Vere frequens in paludosis, foliis gramineis adhaerens'. The following collection in Persoon's herbarium in Herb. L (910. 263–439) may be considered as the type collection: '*Conoplea hispidula* [scr. Pers.] Hb. Pers. [scr. J.H.S.S.] Persoon [scr. ?]' on dead grass leaves. This bears *Periconia hispidula* as redescribed and illustrated by Mason and Ellis (1953). It is interesting to note that Persoon made no mention of spores in this species in 1797 (or later in 1801 and 1822) although it is implied, of course, in the generic diagnosis. This is significant in view of Link's misidentification of the species in 1815 (q.v.).

The three species included in *Conoplea* by Persoon in 1797 show excellent agreement in the abundance of spores (conidia or ascospores) and in the presence of persistent threads (conidiophores or perithecial hairs).

1801. In his Synopsis, Persoon included the three original species of *Conoplea* and added a fourth, *C. atra*, with the following diagnosis, 'Maiuscula subrotunda aut teres atra, puluere parco. Ad ligna locis suffocatis rarius provenit; mollis, 2–3 lin. magna, nonnunquam subeffusa reperitur'.

(4) *Conoplea atra* Pers., Syn. Meth. Fung., p. 235. 1801.
≡*Chaetomium atrum* (Pers.) Link, Linn. Spec. Plant., IV, 6 (1): 46. 1824.

I found no collection on wood identified by Persoon as *Conoplea atra* in his herbarium in Herb. L. However, three other collections so identified have been seen, and all three bear the same fungus:

Herb. L, 910. 263–423. '*Conoplea atra*. Syn. Fung. [scr. Pers.] Hb. Pers. [scr. J.H.S.S.]' on graminicolous stalks and leaves.

Herb. L, 910. 263–433. '*Conoplea atra*. Syn. Fung. *Chaetomium elatum* Kunze et Greville, non rara in cavis cellis aliisque locis suffocatis. [scr. Pers.] Hb. Pers. [scr. J.H.S.S.]', on paper (Fig. 13).

[2]Reference to particular herbaria is made by use of abbreviations found in Index Herbariorum (Regnum Vegetabile, Vol. 6. 1956).
[3]Hereinafter abbreviated J.H.S.S.

Herb. L, 910. 263–435. 'No. 641. *Botrytis*. Ce joli champignon lorsqu'on l'examine au microscope, croit sur le papier qui pourrit. Je l'ai trouvé sur un pot placé à la cave et recouvert de papier [scr. Mougeot] *Conoplea atra* [scr. Pers.] Hb. Pers. ded. Mougeot [scr. J.H.S.S.]' (Fig. 11).

These three collections, each authenticated for *Conoplea atra*, bear *Chaetomium elatum* Kunze.

In 1853 in his Plantes Cryptogames de France (Fasc. II, No. 86), Desmazières issued *Chaetomium elatum* under the name *Chaetomium atrum* (≡*Conoplea atra* Pers.) and added that according to specimens which he had received from Persoon, *Conoplea atra* is a *Chaetomium* and not *Myxotrichum chartarum* as considered by Fries (1832/33) in Systema Mycologicum.

1805. Albertini and Schweinitz included *Conoplea sphaerica*, *C. hispidula*, and two new taxa in their Conspectus fungorum.

(5) *Conoplea atra* var. *caricum* Alb. & Schw., Conspectus fungorum, p. 138. 1805.

(6) *Conoplea betulina* Alb. & Schw., Conspectus fungorum, p. 138. 1805.

I have not seen any of the collections of Albertini and Schweinitz and know of no mention of their whereabouts. Fries (1832/33), in his Systema, included *C. atra* var. *caricum* as a synonym of *Arthrinium puccinioides* (DC.) Kunze. Albertini and Schweinitz also included *C. atra* var. *lignorum* but without a diagnosis.

1805. De Candolle described a new species:

(7) *Conoplea puccinioides* DC., Fl. Française, III, 2: 73. 1805.

≡*Arthrinium puccinioides* (DC.) Kunze in Kunze & Schmidt, Mykol. Hefte, 2: 103. 1823.

≡*Goniosporium puccinioides* (DC.) Link, Linn. Spec. Plant., IV, 6 (1): 45. 1824.

≡*Gonatosporium puccinioides* (DC.) Corda, Icon. Fung., 3: 8. 1839.

I have not seen the type collection but there seems to be no doubt of the correct application of this name; the species is currently classified in *Arthrinium* Kunze.

1809. In his Observationes. . .I, Link described *Exosporium* Link 'Stroma globosum. Sporidia subcylindrica, septata, sessilia, innata' and the single species *E. tiliae* Link 'stromate nigro hirsuto a sporidiis erectis obtusatis nigris. Frequens in ramis mortuis *Tiliae*. . . *Conopleae* nomine mihi quoque missum est'.

The presumed type collection is preserved in Link's herbarium in Herb. B, '*Conoplea* ? sub hoc nomine olim a Persoonio scripsi [scr. ?] *Exosporium tiliae* [scr. Link]'. Two other collections authenticated for *E. tiliae* are:

Herb L, 910. 263–286. '*Exosporium tiliae* [scr. Link] Hb. Pers. [scr. J.H.S.S.]', (Fig. 17).

Herb. Link in Herb. B. '*Sphaeria* [deleted]. . .[deleted] *Exosporium* sphaeriiforme [deleted] *tiliae*. Gregarium globosa. . .atrum. Rost[ock]. [scr. Link] E. Hbr. Link [scr. ?]'.

These three collections bear the same fungus. Two other collections which were identified as *Exosporium* or *Conoplea tiliae* and which bear this fungus are of some interest. They are '*Exosporium tiliae* Link [scr. Fries; on *Tilia*] Herb. E. Fries' (Fig. 14) in Herb. UPS, and '*Conoplea tiliae* Link, Lips. [Leipzig] in ramis tiliaceis [scr. Kunze] Hb. Pers. ded. Kunze [scr. J.H.S.S.]' (Fig. 18) in Herb. L. 910. 263–285.

The fructifications in this fungus (Fig. 1) consist of small erumpent stromata bearing cylindrical, septate, 40–140 μ long, brown conidiophores. The conidia are obclavate, multiseptate, and pale brown, developing from a pore at the apex of the conidiophores. This fungus is congeneric with *Helmisporium velutinum* Link, the type species of *Helmisporium* Link, and may be referred to as *H. tiliae* (Link) Fr. (1832/33, p. 360).

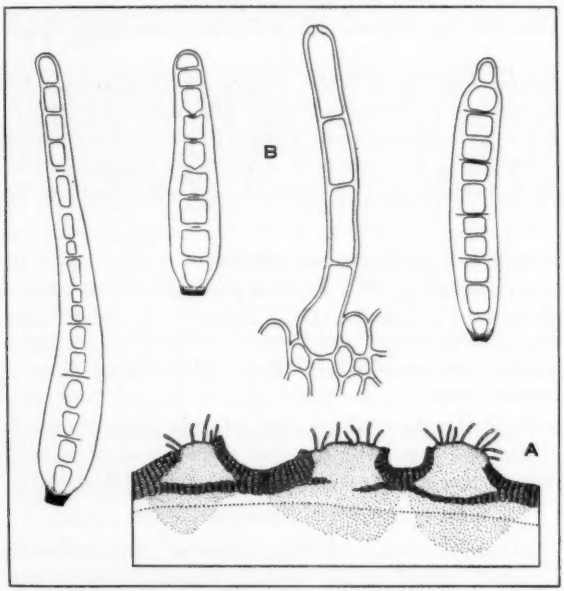


FIG. 1. *Helmisporium tiliae*: A, section through three stromata showing conidiophores on exposed surface, from '*Exosporium tiliae* Lk [scr. Wallr., on *Tilia*]' in Herb. STR, × ca. 60; B, conidiophore and conidia from collection authenticated for *Exosporium tiliae* (Herb. L, 910. 263–286) (cf. p. 66 and Fig. 17), ×500.

From Link's description, illustration, and specimens, it is clear that he did not observe the true conidia; the 'sporidia subcylindrica' of his generic diagnosis must refer to the conidiophores.

The name *Exosporium tiliae* has been applied erroneously for over a hundred years to a totally different fungus (Fig. 2) which has black subspherical stromata, 1–2 mm in diameter, covered by cylindrical brown conidiophores which are only 18–22 μ long and these are continuous or once septate near

the base. The conidia are obclavate, multiseptate, and pale brown to brown, developing on a pore at the apex of the conidiophores. Although the conidiophores are very short and the stromata large I consider this species best called *Helmisporium oligosporum* (Corda) Hughes (1958).

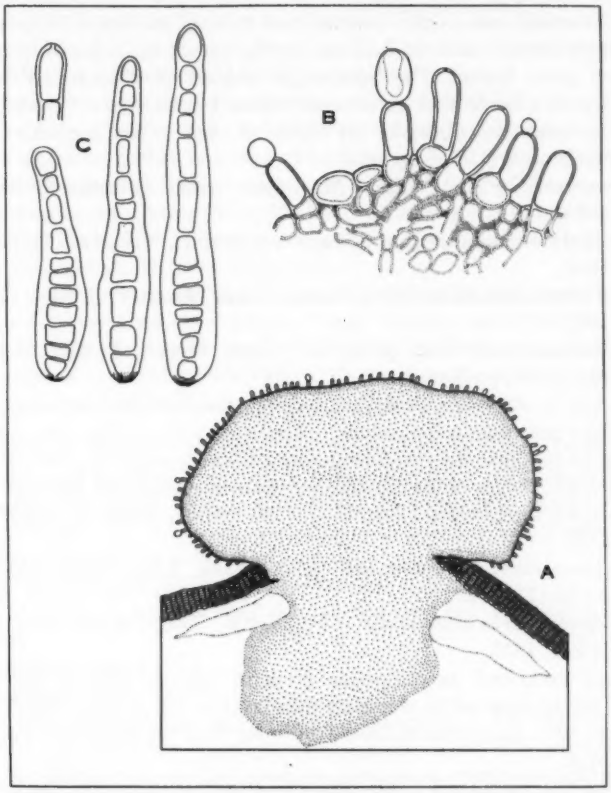


FIG. 2. *Helmisporium oligosporum*, on *Tilia*, Forge Valley, Yorks., England, 16.IX.1946 (Herb. IMI 6787): A, section through stroma showing short conidiophores on the exposed surface, × ca. 60; B, portion of sectioned stroma showing conidiophores and conidium initials, ×500; C, conidiophore and conidia, ×500.

1815. In his Observationes. . .II Link added three species to the genus *Conoplea*; two were new species, *C. hypodermia* and *C. clavuligera*, and the third was his *Exosporium tiliae* of 1809. *Exosporium* was cited as a synonym of *Conoplea* which was given the following much revised diagnosis: 'Stroma globosum aut depressum compactum. Sporidia filiformia saepe septata. [Syn.] *Exosporium*. . . .'

Link included *Conoplea hispidula* in his treatment of *Conoplea* with the following notes, 'subrotunda gregaria insidens atra, sporangiis longissimis

filiformibus. . . In foliis Graminum . . . In his pulverem non conspicio, de quo loquuntur Albertini et Schweinitz. Forte horum planta diversa a Persooniana'. It will be remembered that neither in 1797 nor in 1801 did Persoon mention the presence of 'pulvere' in *C. hispidula* although one would assume this from the generic diagnosis: its presence is also confirmed by an authenticated collection. I have not seen a collection assigned to *C. hispidula* by Link; nevertheless, it seems certain that he had the wrong thing, quite possibly a *Colletotrichum* on grass leaves. The 'sporangiis longissimis filiformibus' of Link's revised diagnosis would then refer to the setae. Furthermore it would account for the inclusion in *Conoplea* by Link of the three species, *C. tiliae*, *C. hypodermia*, and *C. clavuligera*.

(8) *Conoplea tiliae* (Link) Link, Mag. Ges. naturf. Freunde, Berlin, 7: 32. 1815.
≡*Exosporium tiliae* Link, Mag. Ges. naturf. Freunde, Berlin, 3: 10. 1809.
≡ *Vermicularia tiliae* (Link) Schw., Trans. Amer. Phil. Soc., II, 4: 228. 1832.
≡*Helmisporium tiliae* (Link) Fr., Syst. Mycol., 3: 360. 1832/33, as *Helminthosporium.*

Link wrote 'Cum veras *Conopleae* species nunc viderim, certus sum *Exosporium tiliae* non esse separandum quamvis sporidia in hac plantula multo minora ac in reliquis. Recente statu sporidia saepe septata sunt'. Link saw only the conidiophores of his *Exosporium tiliae* and regarded them as conidia.

(9) *Conoplea hypodermia* Link, Mag. Ges. naturf. Freunde, Berlin, 7: 32. 1815.
≡*Exosporium hypodermium* (Link) Link, Linn. Spec. Plant., IV, 6 (2): 121. 1825.
≡ *Vermicularia hypodermia* (Link) Schw., Trans. Amer. Phil. Soc., II, 4: 228. 1832.

This was described as 'elliptica, gregaria sub epidermide prorumpens confluens parva, sporangiis elongatis filiformibus. In ramulis dejectis. . . .' I have not seen an authenticated collection but the diagnosis suggests a *Colletotrichum.*

(10) *Conoplea clavuligera* Link, Mag. Ges. naturf. Freunde, Berlin, 7: 33. 1815.
≡*Exosporium clavuligerum* (Link) Link, Linn. Spec. Plant., IV, 6 (2): 121. 1825.
≡ *Vermicularia clavuligera* (Link) Schw., Trans. Amer. Phil. Soc., II, 4: 228. 1832.
≡*Helmisporium clavuligerum* (Link) Fr., Syst. Mycol., 3: 361. 1832/33, as *Helminthosporium.*

Link described this species as 'subrotunda depressa nigra, sporidiis elongatis filiformibus nigris, aliis clavatis pellucidioribus. In ramis dejectis, sparsa. . . Singularis planta ob sporidia clavata immixta. Misit Dn. Nees ab Esenbeck'. Three collections which I have examined indicate the identity of this species with *Helmisporium tiliae.*

The presumed type collection in Herb. B. is labelled '*Conoplea clavuligera*, Nees ab Esenbeck [scr. Link]' on twig of *Tilia*.

Herb. L, 910. 263–424. '*Conoplea clavuligera* Link ined. [scr. Nees] Hb. Pers. ded. Nees [scr. J.H.S.S.]', on *Tilia*. This may well be a part of the presumed type collection.

'*Conoplea clavuligera* m. Rost[ock]. ad *Salicis* [scr. Link] *Helminthosporium* [Fries, Syst. Mycol.] III. 361 [scr. ?]' (Fig. 23). This is on *Tilia*; it is preserved in Herb. B and is authenticated for *C. clavuligera*.

These three collections bear *Helmisporium tiliae* (Link) Fr. as briefly described above. The diagnosis indicates that Link saw filiform and clavate structures which he called 'sporidia'. The filiform structures are undoubtedly the true conidiophores of this fungus and the clavate ones the true conidia. The same fungus is found on the following collection preserved in the Friesian herbarium in Herb. UPS, '*Exosporium clavuligerum* Nees, Misit Kunze [scr. Fries; on *Tilia*] Herb. E. Fries' (Fig. 21).

To Link in 1815, therefore, *Conoplea* was an assemblage of species with long, dark 'sporidia' produced on a stroma. These lacked completely the 'pulvere manifesto interspersa' which Persoon ascribed to his four species in 1801. Link's inclusion of *C. hispidula* Pers. with such fungi as the foregoing was obviously the result of a misindentification of *C. hispidula*.

1822. Persoon treated *Conoplea* in his Mycologia europaea. Twelve species were distributed between two groups called *Blepharia* and *Conopleae verae*. '*Blepharia*, filis rigidis passim subramosis divergentibus, exsertis. Sporulis paucis aut nullis'. The following species were included in this group:

Conoplea hispidula Pers. (1797)

C. tiliae (Link) Link (1815)

Persoon noted 'Link. . . (qui fila s. fibrillas pro sporulis s. sporidiis considerat)'.

C. clavuligera Link (1815)

C. hypodermia Link (1815)

(11) *C. quercina* Pers.[4] [n. sp.] Mycologia europaea, 1: 11. 1822.

This was described as 'epiphylla fasciculata nigra, filis mollibus. Satis frequens ocurrit in fol. aridis quercinis. *Dematium* refert, sed pulvere nullo adspersa; fasciculus obconicus, e filis pro parvitate satis longis, compositus'. The presumed type collection is in Herb. L, 910. 263–825, '*Conoplea* (*Blepharia*) *quercina* [scr. Pers.] Hb. Pers. [scr. J.H.S.S.] on underside of leaves of *Quercus*. A preparation of this has been examined by Dr. Emil Müller of Zurich, who proposes the combination *Phaeodimeriella quercina* (Pers.) Müller comb. nov.

(12) *C. eryngii* Pers. [n. sp.] Mycologia europaea, 1: 11. 1822.

This species was described thus, 'fasciculis confertis brevissimis atris, filis rigidis, aliis obovatis septatis. Prope Parisios non infrequens ad caules aridos *Eryngii campestris* se propagat, vere. Crustam elongatam primo

[4]In a previous report (Hughes 1958), *C. quercina* Pers. was assigned to *Microxyphium* Auct. The former name was an error for *Fumago quercinum* Pers.

intuitu sistens. Sub lente indagata, observantur filis intermixta corpuscula turbinata; passim subglobosa substipitata, interstitiis distincta. Hinc genus distinctum ex hac specie confici posset, *Elosia* denominandum'.

Three collections pertaining to this name are preserved in Persoon's herbarium in Herb. L: (1) 910. 263–1237, '*Elosia eryngii*, *Conoplea* (*Befaria* [*Blepharia*]) *eryngii*. Prope Parisios. Vere. Caespituli e filis rigidis composita iisque intermixta corpuscula turbinata, passim subglobosa stipitata septata [scr. Pers.] Hb. Pers. [scr. J.H.S.S.]' (Fig. 12). This is undoubtedly the type collection. (2) 910. 263–1224, '*Conoplea eryngii*. Myc. Europ. [scr. Pers.]'. (3) 910. 263–427, '*Elosia eryngii* [scr. Pers.] Hb. Pers. [scr. J.H.S.S.]'.

These three collections bear an *Alternaria* Nees and the combination *A. eryngii* (Pers.) Hughes and Simmons was made in Hughes (1958). The suggested generic name *Elosia* of Persoon has never been validly published.

There are preserved in Herb. E. Fries, in Herb. UPS, specimens from France labelled '*Conoplea eryngii*' in an unidentified handwriting. These bear effete pustules of a *Colletotrichum* together with some conidia of *Alternaria* and *Cladosporium*. Fries cited a collection from France in the Systema; his diagnosis may well have been based on the French specimens.

'*Conopleae verae*, caespitulis mollibus, filis intertextis subfloccosis, pulvere sat copioso refertis'. The following species were included in this group.

C. sphaerica (Pers.) Pers. (1797)

(13) *C. fusca* Pers. [n. sp.] Mycologia europaea, 1: 12. 1822.

This was described briefly as 'subrotunda confluensque fusca'. The type collection has been examined; *C. fusca* is congeneric with *C. sphaerica* and is described and illustrated on pp. 683–687. *C. fusca* is an earlier synonym of *Streptothrix fusca* Corda, the type species of *Streptothrix*.

(14) *C. cinerea* Pers. [n. sp.] Mycologia europaea, 1: 12. 1822.

This was described briefly as 'subrotunda confluensque cinerea. Rarius prope Parisios ad ramos aridos inveni. 3–4 lin. lata'. The presumed type collection has been examined in Herb. L, '910. 263–425. *Conoplea cinerea* [scr. Pers.] Hb. Pers. [scr. J.H.S.S.]'. This bears the *Gonytrichum* conidial state of *Melanopsammella inaequalis* (Grove) Höhnel (=*Gonytrichum caesium* Nees & Nees).

(15) *C. gilva* Pers. [n. sp.] Mycologia europaea, 1: 12. 1822.

This species was described as 'sparsa subcompacta ex gilvo subrufescens. Ad caules herbarum majorum passim inventa a D. Cauvin conferti'. Two collections identified by Persoon are preserved in Herb. L.

The type collection, 910. 263–431, 'tige de plante herbacée—Pontivy [scr. Cauvin] *Conoplea? gilva* [scr. Pers.] Hb. Pers. [scr. J.H.S.S.]' (Fig. 19). The second collection is 910. 263–1238, '*Conoplea* gilva? Myc. Europ. An potius *Sporotrichum*?. Prope Parisios [scr. Pers.]'. Both collections bear *Sarcopodium circinatum* Ehrenb. The following collection in Herb. UPS also bears *S. circinatum*, '*Conoplea gilva* P. Scania [scr. Fries; on herbaceous stem] Herb. E. Fries,' (Fig. 22).

C. atra Pers. (1801)

Persoon questioned the generic disposition of this species as follows 'Confiteri debeo, quod specimina, Parisiis collecta, obtulerunt sphaeriae speciem, *Sph.* Byssisedae similem, villo obvolutam, an forte parasiticam? cum simul pulvere digitos inquinante scatet. Hinc nisi *Sphaeriae* sint, ad genus *Chaetomium* Kunze referri debet'. Figure 13 indicates that Persoon had added '*Chaetomium elatum*' to the label of one of his *Conoplea atra* collections.

C. cylindrica Pers. (1797)

Persoon added the note 'A priore [*C. atra*] forte non specie distincta'.

This was Persoon's final treatment of *Conoplea*. Following an examination of type and authenticated material it is now known that Persoon's nine species belong in a number of different genera; their disposition may be summarized thus:

C. sphaerica	(1797)	≡ lectotype species of *Conoplea*
C. hispidula	(1797)	≡ *Periconia hispidula* (Pers.) Mason & Ellis
C. cylindrica	(1797)	= *Chaetomium elatum* Kunze
C. atra	(1801)	= *Chaetomium elatum* Kunze
C. quercina	(1822)	≡ *Phaeodimeriella quercina* (Pers.) Müller
C. eryngii	(1822)	≡ *Alternaria eryngii* (Pers.) Hughes & Simmons
C. fusca	(1822)	≡ *Conoplea fusca* Pers.
C. cinerea	(1822)	= *Gonytrichum* state of *Melanopsammella inaequalis* (Grove) Höhnel
C. gilva	(1822)	= *Sarcopodium circinatum* Ehrenb.

1822. Schweinitz described his new species

(16) *Conoplea ferruginea* Schw., Schr. naturf. Gesellsch. Leipzig, 1: 77. 1822. ≡ *Torula ferruginea* (Schw.) Spreng., Linn. Syst. Veg., XVI, 4 (1): 556. 1827.

The diagnosis is as follows, '*C.* sparsa majuscula, pulvere ferrugineo-purpureo subpulvinato. In asseribus non rara. — Video fila brevia rigida in articulos sphaeroides apice et basi compressos, fere opacos secedentia. An *Torula*?'. There is no collection of this preserved in Herb. PH or in Herb. BPI.

1824. Link had discovered his erroneous identification of *Conoplea hispidula* before he wrote up the Fungi in 1824. In this work he restricted *Conoplea* to the one species *C. hispidula* and indicated the presence of 'sporidia circa basin floccorum inspersa simplicia (non septata)'. Link may well have learned this fungus correctly from Kunze and Schmidt, Deutschlands Schwämme No. 69 sub *C. hispidula*; at least he cited this exsiccatum in 1825. Link made the following comments: '*Conopleae* aliae species ab Auctoribus indicantur, quae aut hujus loci non sunt, aut accurate non indicatae *C. tiliae, clavuligera, hypodermia* Pers. ad *Exosporium* pertinent. *C. quercina* ej. ignota, nec non *C. eryngii* et *C. sphaerica* ej. *C. fusca* ej. a Dr. Kunze ipsi et mihi nomino *Helminthosporii subramosi* communicata est *Oidium subramosum. C. cinerea, gilva*, et *cylindrica* ej. ignotae. *C. atra* ej. est *Chaetomium atrum. C. betulinum* Albert. et Schwein. nisk. p. 138 non vidi. *C. puccinioides* de Cand. fr. 2. p. 73. videtur *Arthrinium caricicola* Schmidt et Kze'.

Link here reinstated his generic name *Exosporium* for *E. tiliae*, *Conoplea clavuligera*, and *C. hypodermia*. At the same time he recognized *C. atra* as a *Chaetomium*; this is confirmed by a recent examination of authenticated material.

1825. In the continuation of his treatment of Fungi, Link (1825) gave the following revised diagnosis for *Exosporium*, 'Macula effusa. Sporidochium verruciforme. Sporidia sessilia septata. Not. Ad *Conopleas* accedit sed sporidochium sat conspicuum et qui flocci innati videntur evidenter septati et sporidiis aliis globosis minime intermixti, tum apice non truncati sed attenuati et rotundati seu clausi sunt, hinc sporidia vocanda. A Sphaeriis rostratis septis sporidiorum dignoscendum'.

Link included the following species in his reformed *Exosporium*:

E. tiliae Link (1809): this is the original species.

E. clavuligerum (Link) Link ≡ *Conoplea clavuligera* Link (1815).

E. hypodermium (Link) Link ≡ *Conoplea hypodermia* Link (1815) ≡ *Vermicularia hypodermia* (Link) Schw. (Trans. Amer. Phil. Soc., II, 4: 228. 1832). I have not seen the type collection but Link's expanded diagnosis certainly suggests a *Colletotrichum*.

E. hispidulum Link ≡ *Vermicularia hispidula* (Link) Schw. (loc. cit.). This taxon is based upon that collection which Link erroneously assigned to *Conoplea hispidula* Pers. in 1815. I have not seen the type collection but the diagnosis suggests a *Colletotrichum*. *Exosporium hispidulum* Link is a later homonym of *E. hispidulum* (Pers.) Nees (1816/17) ≡ *Conoplea hispidula* Pers.

E. dematium (Pers.) Link ≡ *Sphaeria dematium* Pers. (Syn. Meth. Fung., p. 88. 1801) ≡ *Vermicularia dematium* (Pers.) Schw. (loc. cit.) ≡ *Colletotrichum dematium* (Pers.) Grove (J. Bot., Lond., 56: 341. 1918). I have not seen the type collection but so far as I am aware its disposition as a *Vermicularia* or *Colletotrichum* has never been doubted.

E. minutum Link ≡ *Sphaeria dematium* Pers. β *minor* Fr. (Syst. Mycol., 2: 505. 1823) ≡ *Vermicularia minuta* (Link) Schw. (loc. cit.) ≡ *Colletotrichum minutum* (Link) Dickson (Mycologia, 17: 216. 1925). According to von Arx (1957, p. 458) this is identical with *Colletotrichum dematium* (Pers.) Grove. I have examined the Herb. FH copy of the type collection of *Exosporium minutum*, which is Fries, Scleromyceti Sueciae, 53, sub *Sphaeria dematium* β *minor*, and found effete acervuli of a *Colletotrichum*.

E. trichellum (Fr.) Link ≡ *Sphaeria trichella* Fr. (K. Vet. Acad. Handl., 38: 256. 1817) ≡ *Sphaeria dematium* Pers. β *capraea* Pers. (Syn. Meth. Fung., p. 88. 1801) ≡ *Vermicularia trichella* (Fr.) Fr. in Greville (Scot. Crypt. Flora, 6: 345. 1828) ≡ *Amerosporium trichellum* (Fr.) Lind (Danish Fungi, p. 471. 1913) ≡ *Colletotrichum trichellum* (Fr.) Duke (Trans. Brit. Mycol. Soc., 13: 173. 1928). *Sphaeria trichella* Fr. is based on '*Sph. Dematium* β. Pers. syn. p. 88' as clearly indicated by Fries in 1817. I have not seen Persoon's type which is on *Salix capraea*. Fries' Scleromyceti Sueciae, 203 anno 1821, issued as *Sphaeria trichella* is on *Hedera* and bears a *Colletotrichum*. Von Arx (1957, p. 458) included *C. trichellum* as a synonym of *C. dematium* (Pers.) Grove.

E. ditrichum (Fr.) Link≡*Sphaeria ditricha* Fr. (Syst. Mycol., 2: 515. 1823) ≡ *Vermicularia ditricha* (Fr.) Fr. (Summa Veg. Scan., 2: 420. 1849)≡ *Venturia ditricha* (Fr.) Karst. (Mycologia Fennica, Part 2 (Pyrenomycetes), p. 188. 1873). The Herb. FH copy of the type collection (Fries, Scleromyceti Sueciae, 54, anno 1820) bears a *Venturia*.

E. rubi Nees. According to Fries (1828, p. 125) this is a young state of *Dothidea chaetomium* Kunze in Fr. (Syst. Mycol., 2: 563. 1823)≡*Coleroa chaetomium* (Kunze) Rabenh. I have not seen the type collection.

E. maculans Link≡*Vermicularia maculans* (Link) Schw. (loc. cit.)≡ *Colletotrichum maculans* (Link) Dickson (Mycologia, 17: 214. 1925). This is *C. dematium* (Pers.) Grove according to von Arx (1957, p. 458). I have not seen the type collection.

1826. de Brondeau renamed *Conoplea cylindrica* Pers. as *C. comosa*; his illustrations and discussion indicate that he undoubtedly had *Chaetomium elatum* Kunze (=*Conoplea cylindrica*) under consideration. He drew attention to the resemblance of his specimens with *Sphaeria comata* Tode (1791) which Chivers (1915, p. 181) regarded as *Chaetomium elatum*.

1827. Sprengel, with the following new combination, added a seventeenth name into *Conoplea*:

(17) *Conoplea elata* (Kunze) Sprengel, Linn. Syst. Veg., XVI, 4 (1): 554. 1827.

≡*Chaetomium elatum* Kunze in Kunze & Schmidt, Deutschl. Schwämme, 8: p. 3, No. 184. 1818.

This disposition is interesting in view of the fact that both *Conoplea cylindrica* and *C. atra* are identical with *Chaetomium elatum*. So far as I am aware, this is the first and only time that a *Chaetomium* was deliberately transferred to *Conoplea*.

1832. Schweinitz described 24 new species of *Vermicularia* and transferred to this genus most of the species that Link had compiled in *Exosporium* in 1825. Schweinitz remarked 'Setae quibus semper gaudet, pro Sporidia habet Link, vix jure'. Persoon made a similar comment in 1822. *Exosporium tiliae* and *Conoplea clavuligera* were also transferred to *Vermicularia* but Schweinitz questioned the correctness of the disposition thus; 'An duae primae species [*E. tiliae* et *C. clavuligera*] ad hoc genus pertineant, an genus proprium—nomine *Exosporium* designandum et loco Linkii inter Gymnomycetes locandum efficient—adhuc incertus sum'.

1832/33. Fries included '*Conoplea* (Pers.) Fries nec Auct.' in his Systema with the following diagnosis, 'Sporidia in floccos moniliformes concatenata et in tuberculum compactum, extus conidiis adspersum, conglobata: sporidiorum simplicium catenis demum secedentibus'.

Fries included three of Persoon's names in *Conoplea*; *C. cinerea*, *C. sphaerica* (Fries altered the epithet to *olivacea*), and *C. fusca*. From Persoonian collections it is now known that the last two species are congeneric but *C. cinerea* is a

Gonytrichum. There are no collections of *C. cinerea* in Fries' herbarium in Herb. UPS or in Herb. LD, but it is evident from the description that Fries must have had a fungus quite different from that of Persoon. This is understandable in view of the three-word diagnosis by Persoon and about which Fries commented 'quantum quidem e tribus verbis, externam faciem spectantibus, concludi potest'. Fries preserved no collection of *Conoplea fusca* but a gathering of what he considered to be *C. olivacea* (≡*C. sphaerica*) is to be found in Herb. UPS and was also issued by him in Scleromyceti Sueciae, No. 439. This is on twigs, very probably of *Salix* according to Professor J. A. Nannfeldt, but the fungus is not Persoon's *C. sphaerica* and it is not congeneric with any other species assigned to *Conoplea* by Persoon.

Fries disposition of names assigned to *Conoplea* is as follows:

C. sphaerica (Pers.) Pers. (1797)	≡*Conoplea olivacea* Fr. (nom. nov.)
C. cylindrica Pers. (1797)	≡*Psilonia cylindrica* (Pers.) Fr.
C. hispidula Pers. (1797)	≡*Dematium hispidulum* (Pers.) Fr.
C. atra Pers. (1801)	= *Myxotrichum chartarum* (Nees) Kunze
C. betulina Alb. & Schw. (1805)	(not mentioned)
C. atra var. *caricinum* Alb. & Schw. (1805)	= *Arthrinium puccinioides* (DC.) Kunze
C. puccinioides DC. (1805)	≡*Arthrinium puccinioides* (DC.) Kunze
C. tiliae (Link) Link (1815)	≡'*Helminthosporium*' *tiliae* (Link) Fr.
C. hypodermia Link (1815)	= *Vermicularia*
C. clavuligera Link (1815)	≡'*Helminthosporium*' *clavuligerum* (Link) Fr.
C. quercina Pers. (1822)	('multis convenit'—Index).
C. eryngii Pers. (1822)	≡'*Helminthosporium*' *eryngii* (Pers.) Fr.
C. fusca Pers. (1822)	≡*Conoplea fusca* Pers.
C. cinerea Pers. (1822)	≡*Conoplea cinerea* Pers.
C. gilva Pers. (1822)	≡*Psilonia gilva* (Pers.) Fr.
C. ferruginea Schw. (1822)	(briefly mentioned on p. 429)
C. elata (Kunze) Sprengel (1827)	≡[*Chaetomium elatum* Kunze]

The name *Conoplea alba* is mentioned by Fries (1832/33, p. 490) in his observations on the genus *Conoplea*. So far as I am aware the name is based on Nees' collection labelled *Dichosporium aggregatum* Nees (1816/17, p. 105). *D. aggregatum* is a new name for *Spumaria physaroides* Pers., which was cited as a synonym by Nees. Fries (1829, p. 219) compiled *Dichosporium aggregatum* but added 'A *Spumaria physaroide* Pers. diversum habeo'; Nees' collection of *D. aggregatum* is preserved in Herb. Fries in Herb. UPS and Fries recorded having seen it '(v.s. a Nees communicat.)'. Later, Fries (1832/33, p. 490) wrote 'Denique observandum diu mihi dubium genus, *Dichosporium* Nees, Syst. Myc. III. p. 218., vix non hujus esse speciem, licet character et icon diversam monstrent indolem; specimen, quod adest, ad examen non quidem suffecit, at ni valde fallor, *Dichosp. aggregatum* Nees Syst. f. 99. *Conoplea alba* olim erit dicendum'.

Nees' specimen, from which his illustration was drawn, consists of sporangia of a Myxomycete which have a white outer layer of *Verticillium* conidiophores and conidia. The name *Conoplea alba* is at best a nomen confusum.

1837. F. F. Chevalier published the generic name *Streblocaulium* for the single species *S. atrovirens* Chev., 'Hanc speciem inveni autumno ad corticem rami exsiccati *Carpini* in sylva Kastewald in Alsatia prope Colmariam urbem'. I have failed to trace the type collection but what indicates very strongly that Chevalier had a *Conoplea* (? *sphaerica*) are (1) his choice of generic

name derived from '*στρεβλος*' and '*χαυλος*'—twisted stem, (2) his illustrations, and (3) particularly certain parts of his description 'Les filamens . . . très-rameux et remarquables en ce que leurs rameaux sont flexueux ondulés. . . sporidies . . . naissent aux extrémités et à l'endroit des cloisons des rameaux . . . . L'on remarquera aussi parfois des rameaux courts, disposés en une rosette. . . ou l'on trouvera des sporidies gèminées à l'extrémité d'un court pédicelle légèrement recourbé mais qui ne se prolonge pas au delà en une sorte de corne'.

1839. Corda (1839, p. 27) published the generic name *Streptothrix* for the single species *S. fusca* Corda which, in the French edition of this work, was stated to occur 'sur les branches écorcées de la rose sauvage (*Rosa canina*), dans le jardin du prince de Lobkowitz, à Prague, et pendant l'automne de 1837'. I have not seen the type collection; it is not preserved in Herb PR, but Corda's illustration and description leave no doubt in my mind that he was dealing with *Conoplea fusca* Pers.

Like so many polytypic classical genera *Conoplea* was mixed from its origin in 1797. By 1827, successive treatments and additions had resulted in 16 species and a variety being assigned to the genus; these represent probably 12 different genera. It is understandable that the name has fallen into disuse and that it has been described as a *nomen dubium* in 'A dictionary of the Fungi' by Ainsworth and Bisby. Resource to type and authenticated collections, however, enables the original species to be explained.

As delimited in this publication, with *Conoplea sphaerica* as lectotype species. *Conoplea* comprises a small genus of economically unimportant molds although one of the species, *C. globosa* (under the name *Strumella coryneoidea*), has been the subject of a number of publications because of its apparent cause of cankers of a number of tree species, especially of *Quercus* and of *Castanea* in North America. *Conoplea* replaces *Streptothrix* Corda (1839) but Corda's generic name would in all probability have to give way to the earlier *Streblocaulium* Chev. (1837).

If *Conoplea cylindrica* (= *Chaetomium elatum*), the second of the three original species of *Conoplea*, were chosen as lectotype species, then *Conoplea* would become the earliest *effectively* published generic name for *Chaetomium* as currently employed. If *Conoplea hispidula*, the third of the original species, were chosen as lectotype species, then *Conoplea* would presumably be a synonym of *Periconia* Tode. However, the identity of the original species of Tode's generic name, *P. lichenoides* Tode, is unknown, but the name *Periconia* is being widely used for species congeneric with *Conoplea hispidula*.

Taxonomic Part

CONOPLEA Pers., Tent. Disp. Meth. Fung., p. 55, 1797, Syn. Meth. Fung., p. 234, 1801; also in Fries, Syst. Mycol., 3: 490. 1832/33.

Lectotype sp., *C. sphaerica* (Pers.) Pers. (≡ *C. olivacea* Fr.)

?= *Streblocaulium* Chev., Fungorum et Byssorum Illustrationes, Tome 1. 1837.

Type sp., *S. atrovirens* Chev. = ? *Conoplea sphaerica* (Pers.) Pers.
= *Streptothrix* Corda, Pracht-flora. . .p. 27. 1839.

Type sp., *S. fusca* Corda = *Conoplea fusca* Pers.

Conoplea species form brown fructifications which may be pulvinate or flattened in the form of a turf or may consist of solitary or groups of a few conidiophores scattered on the substratum.

The mycelium is usually immersed, sometimes superficial, and may form erumpent pulvinate stromatic aggregations on corticated woody twigs. Superficial and creeping hyphae are formed by one species on bare wood.

Conidiophores are pale brown to dark brown, septate, rough-walled, and may be helically twisted; they are generally arborescent and the branching may be more or less irregular or a well-differentiated main stalk is evident with primary, secondary, and sometimes tertiary branches. Towards the base, conidiophores may show frequent anastomoses with neighboring conidiophores. Specialized sporogenous branchlets are produced in some species; these develop laterally and are rarely cut off from the cell that bears them. They are generally short and adpressed to the conidiophore. In two species at least, cells of the mature conidiophore may elongate by intercalary growth and may become detached, one from the other; the result is a pustule of conidiophore fragments and conidia.

Conidia are terminus spores produced singly as the blown-out end of each successive new growing point which develops just to one side of the previous terminal conidium which is itself pushed to a lateral position by the new developing conidium. They develop on short specialized sporogenous branchlets or on undifferentiated branches, and finally on the terminal cells of the arborescent conidiophore or conidia may develop directly on a sparingly branched conidiophore. During development, a thin disk-like separating cell may be differentiated between conidium and conidiophore or branchlet. Conidia fall readily from the conidiophore and form a dark-brown to black deposit. They are dry, powdery, subglobose to pyriform, or oval and occasionally angular, small (ca. 6–12 μ), pale brown to dark brown, inconspicuously or coarsely roughened, with a scar on a slightly or well raised base which may show a marginal frill of wall material. Conidia have a hyaline, longitudinal (?)germination slit, or a circular, terminal or lateral, thin-walled area (?germination pore).

Habitat: on bare wood, corticated branches and leaves of conifers, branches of broad-leafed trees, canes of *Rubus* and *Rosa*, woody herbaceous stems, and leaves of *Carex*.

Key to Species and Varieties of *Conoplea*

Conidia with longitudinal germination slit
  Conidiophores up to 1300 μ long, with well-differentiated main stalk, never breaking up into component cells.....*sphaerica* (p. 673)
  Conidiophores up to 650 μ long, with poorly differentiated main stalk, often breaking up into component cells.....*globosa* (p. 679)
Conidia with germination pore
  Conidiophores helically twisted

Pore lateral, usually just above conidium scar.....*fusca* (p. 683)
Pore apical or subapical
Conidia mostly 6–7.5×4.7–5.8 μ.....*juniperi* var. *juniperi* (p. 687)
Conidia 7–13.5×5.6–9.5 μ.....*juniperi* var. *robusta* (p. 690)
Conidiophores not helically twisted
Conidia on definite sporogenous branchlets.....*geniculata* (p. 691)
Conidia directly on conidiophores.....*abietina* (p. 694)

Description of Species and Varieties

Conoplea sphaerica (Pers.) Pers., Tent. Disp. Meth. Fung., p. 55. 1797, and Syn. Meth. Fung., p. 234. 1801 (Fig. 3).

≡ *Dematium sphaericum* Pers., Usteri's Ann. Bot., 15 Stück: 25. 1795.

≡ *Conoplea olivacea* Fr., Syst. Mycol., 3: 491. 1832/33.

? = *Streblocaulium atrovirens* Chev., Fungorum et Byssorum Illustrationes, Tome 1. 1837.

= *Acremonium velutinum* Fuckel, Symb. Mycol., Nachtr. II. p. 79. 1873.

≡ *Acremoniella velutina* (Fuckel) Sacc., Syll. Fung., 4: 303. 1886.

= *Trichosporum densum* Karst., Hedwigia, 23: 59. 1884 (April) and Meddel. Soc. F. F. Fennica, 11: 143. 1884, as *Trichosporium.*

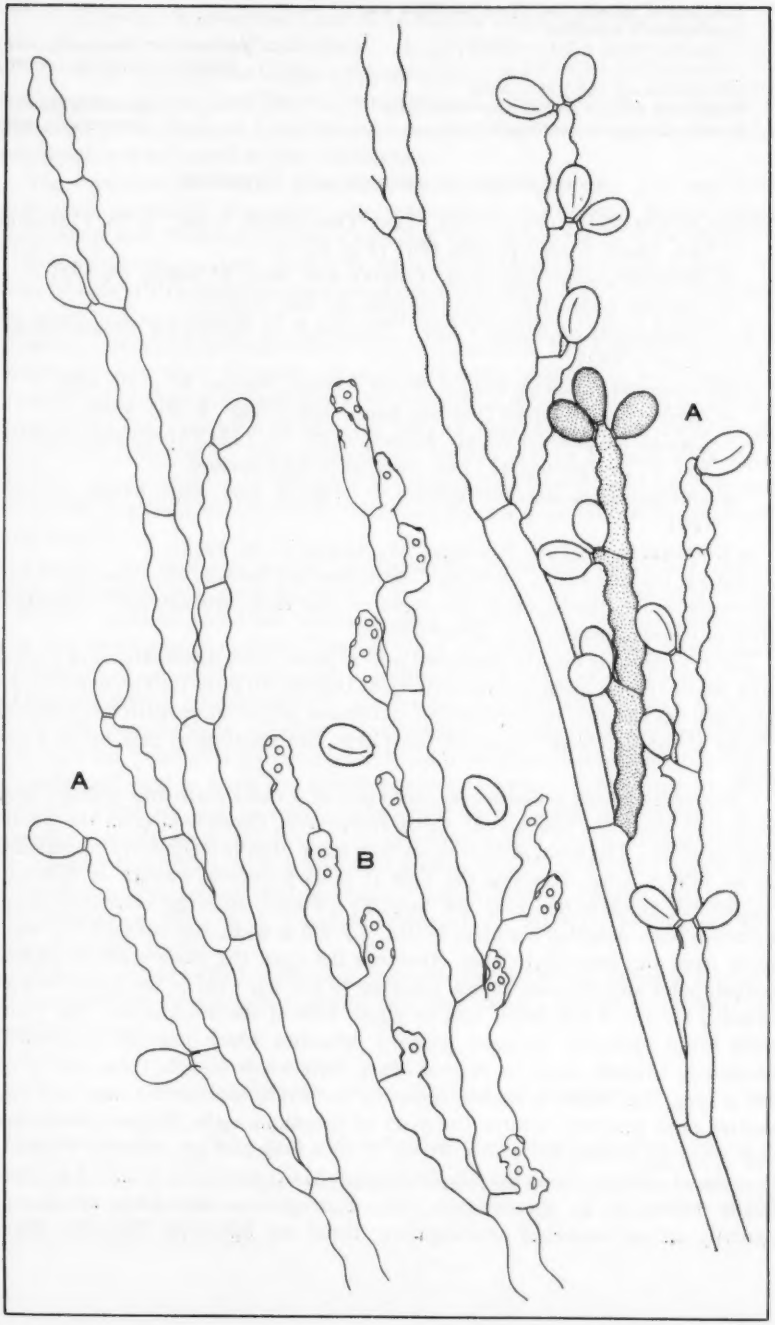
≡ *Colletosporium densum* (Karst.) O. Kuntze, Rev. Gen. Plant., 2: 849. 1891.

= *Streptothrix pereffusa* Sumstine, Mycologia, 6: 34. 1914.

Mature fructifications on woody branches are brown, dry and powdery, pulvinate and scattered, or so close together that they form extensive unbroken mats of conidiophores 5 cm or more wide.

The mycelium is mostly immersed and in most cases aggregations of fungal cells below the periderm eventually burst through to form pulvinate stromata on which conidiophores are produced. Stromata produced beneath longitudinal cracks in the periderm, especially on *Carya*, are continuous and up to 5 cm long.

Conidiophores are arborescent, composed of a main stalk and primary and secondary branches which bear sporogenous cells; they develop on the pulvinate stromata. The main stalk is straight or more usually irregularly curved and up to 1300 μ long. Towards the base it is dark brown, smooth or sparsely roughened, thick-walled and not helically twisted, showing occasional anastomoses with neighboring main stalks, 4.5–6.5 μ wide, but up to 7.5 μ wide away from the immediate base. Towards the apex the main stalk is rough-walled, paler and thinner-walled, tapering to 3.8–4 μ wide at the apex; helical twisting occurs in the upper half to upper fifth of the main stalk. The main stalk bears upwardly directed primary branches which may be cylindrical below and twisted above or twisted along their whole length; these are up to 300 μ long, the longer branches generally occurring nearest the base and the shorter ones generally nearer the apex of the main stalk. Primary branches bear secondary branches which are up to 90 μ long and are helically twisted. Occasional tertiary branches are developed. Main stalk and lateral branches finally terminate in sporogenous cells. Sporogenous branchlets are borne laterally on the branched conidiophore; these are upwardly directed, often


A
B
A

closely adpressed to the branch that bears them; they are straight or curved, subcylindrical and irregular in outline with denticulate conidium scars, tapering somewhat towards the apex, and they measure 8–13×3.4–4 μ.

Conidia are terminus spores and are produced on the sporogenous branchlets and on the fertile ends of the main stalk and its branches. During development of the conidium, a thin (0.5–1 μ) hyaline separating cell is differentiated. Conidia are oval, somewhat narrowed towards the base, infrequently subspherical, with an inconspicuous basal scar, sometimes with a marginal frill, dark brown, coarsely roughened, with a narrow hyaline longitudinal (?) germination slit; they measure 6–11(–16)×4.7–5.5(–6.5) μ.

Hosts: *Acer*, *Asimina*, *Betula*, *Carpinus*, *Carya*, *Cornus*, *Corylus*, *Crataegus*, *Fagus*, *Fraxinus*, *Hamamelis*, *Juglans*, *Nemopanthus*, *Ostrya*, *Prunus*, *Quercus*, *Ribes*, *Taxus*, *Tilia*, *Ulmus*.

Geographical sources of collections: Canada (Ont., Que.), U.S.A. (N.Y., N.H., Pa., Conn., Va., W.Va., Ohio, Iowa, Ill., Ind., Mich., Wisc., Mo.), Europe (France, Germany, Latvia, Poland, Sweden, Finland, U.S.S.R.).

Collections Examined

Type and authenticated collections, published exsiccati, and some classical collections:

(1) The presumed type collection of *Conoplea sphaerica* Pers. (Fig. 9), '*Conoplea sphaerica*. Syn. fung. [scr. Pers.; on bark] Hb. Pers. [scr. J.H.S.S.]', in Herb. L (910. 263–287).

(2) The lectotype collection of *Acremonium velutinum* Fuckel, Fuckel, Fungi Rhenani (Suppl.), '2590. *Acremonium velutinum* Fckl. Ad *Carpini Bet.* ramos corticatos putridos, raro. Hieme. Ca. Eberbach,' in Herb. FH. A distribution ex 'Herbier Barbey-Boissier' of Fuckel's material under the number 2553 and the name *Acremoniella velutina* (Fuckel) Sacc. is on *Carpinus betulus* from Eberbach and is very probably part of this collection. The second collection cited by Fuckel (1873, p. 79) was from Bachweg, but I have not seen this. A collection in 'Herbier Barbey-Boissier' in Herb. G, labelled with a cutout of the diagnosis from Fuckel (1873), bears *Conoplea sphaerica* but it is not indicated whether the collection is from Eberbach or Bachweg.

(3) The presumed type collection of *Trichosporum densum* Karst., '*Trichosporium densum* Karst. [on a twig], [conidia] ovoidea fuliginea, hyphae fuligineo-pallidae, diaphanae [scr. Karst.] Herb. P.A. Karsten', in Herb. H.

(4) Collection authenticated for *Trichosporum densum* Karst., '*Trichosporium densum*? ad *Tiliam*, Gallia [scr. Hariot], *Trichosporium densum* Karst! (ex ipso) Géverdot (Aube), Sept. '89 [scr. Hariot]'. Herb. PC. The previous collection in Herb. H may well be a part of this one.

(5) The type collection of *Streptothrix pereffusa* Sumstine, 'Bernus Point [N.Y.], July 1913, David R. Sumstine. Part of original collection', DAOM 36859.

FIG. 3. *Conoplea sphaerica*: A, portions of two young conidiophores, and conidia from collection No. 18 (p. 676); B, portion of old conidiophore, and conidia from collection No. 17. (p. 676). ×1000

(6) Reliquiae Farlowianae, 202*a*. *Streptothrix atra*, Chocorua, N.H., on *Acer*, Sept. 1906. W. G. Farlow. DAOM 29011. No. 202*b* in this series bears *Conoplea globosa*.

(7) Transchel et Serebrianikow, Mycotheca Rossica, 50. *Streptothrix fusca*. Schebekino prov. Kursk, on *Corylus avellana*, 16.VIII.1908. Herb. Dearness.

(8) J. Smarods, Fungi Latvici exsiccati, 148. *Streptothrix fusca*. 'Prov. Kurzeme, Kr. Ventspils: Usma, auf der Insel Moricsala in Usmas ezers', on *Corylus avellana*, 5.VI.1931. K. Starcs. Herb. BPI.

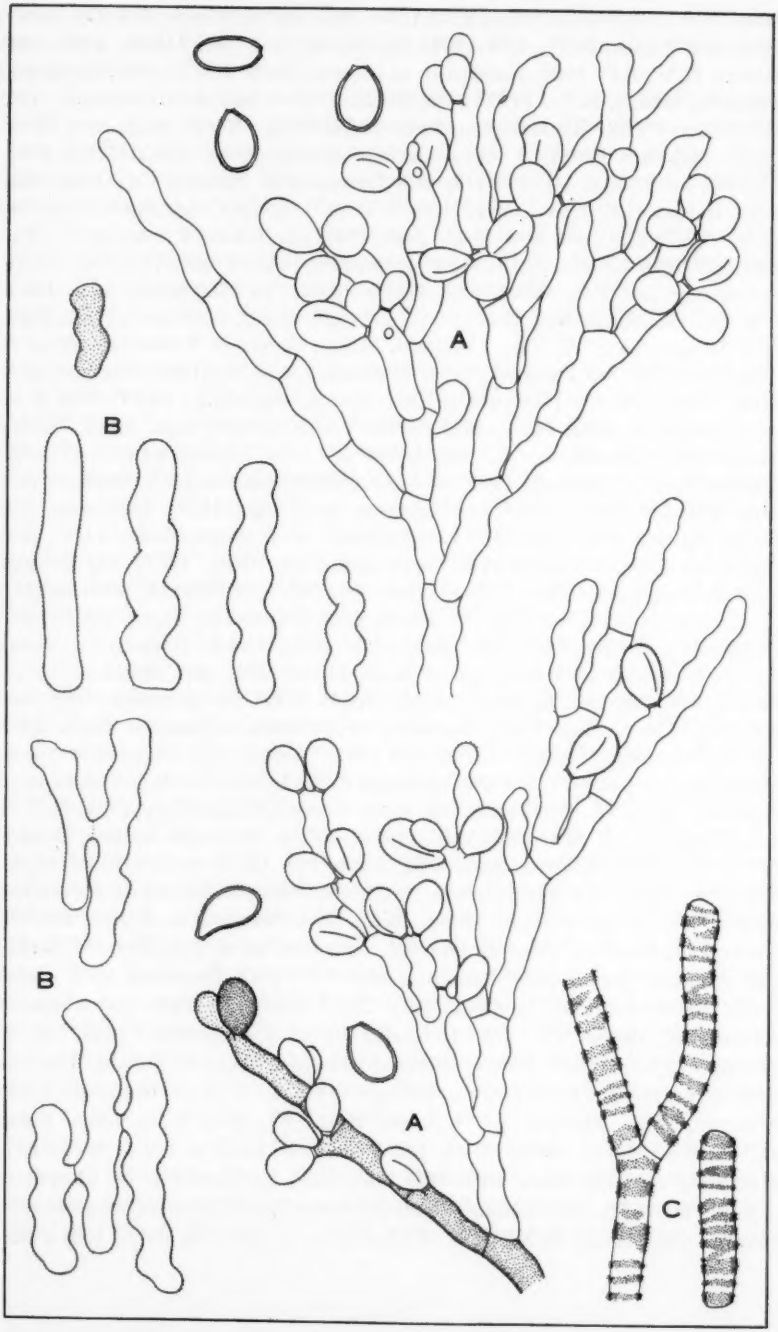
(9) '*Virgaria nigra* ? N.E. in *Corylo* [scr. Fries] Herb. E. Fries'. Herb. UPS.

(10) '3827. Pennsylv. Michener [scr. Berk.]'. Herb. K. (*Streptothrix atra* folder). This is one of the cotype collections of *S. atra* (cf. p. 688).

(11) '*Trichosporium fuscum* (Link) Sacc. [on bark] Millardi. Pazschke [scr. Sacc.]'. Herb. PAD.

Other collections: on ACER: (12) Chalk River, Ont., 31.VIII.1941, Jackson, as *S. atra*, Herb. TRT; (13) Inlet, N.Y., 23.VIII.1934, Conners, as *S. fusca*, DAOM 2272. On ASIMINA: (14) ?Preston, Ohio, 27.X.1892, Morgan, as *S. fusca*, Herb. IA. On BETULA: (15) Buckingham, Que., 26.IX.1903, Macoun, as *S. atra*, Herb. TRT. On CARPINUS: (16) London, Ont., June 1894, Dearness, as *S. atra*, Herb. BPI; (17) E. Spits [near London, Ont.], Nov. 1913, Dearness, as *S. atra*, Herb. Dearness; (18) Chelsea, Que., 26.IX.1902, Macoun, as *S. atra*, DAOM 34510. On CARYA: (19) Gatineau Hills, near Hull, Que., 4.X.1956, Hughes, DAOM 51661; (20) River Forest, Ill., 19.IV.1902, Harper, as *S. atra*, DAOM 36863; (21) Riverside, Ill., 13.III.1909, E.T. & S.A. Harper, as *S. atra*, Herb. NY; (22) Lafayette, Ind., 27.X.1888, Bolley, as *S. atra*, Herb. NY; (23) Reitz Gap, Center Co., Pa., 10.III.1925, Overholts (9719), as *S. atra*, Herb. Dearness; (24) Ann Arbor, Mich., 26.X.1934, Wehmeyer (442), as *S. pereffusa*, Herb. NY, (25) Ann Dane, Dane Co., Wisc., 21.IV.1953, Greene, as *S. fusca*, Herb. WIS. On CORNUS: (26) near Tetreauville, Que., 2.XI.1898, Macoun, as *S. atra*, DAOM 36864. On CORYLUS: (27) Gull Lake Portage, L. Temagami, Ont., 10.IX.1932, Groves, as *S. fusca*, Herb. FH; (28) [Minaki, Ont.] Bisby (4129), as *S. ?fusca*, Herb. Dearness; (29) W. Tripoli, N.Y., 13.V.1917, Burnham (205), as *S. atra*, Herb. Dearness; (30) Esbo, Finland, 13.X.1908, Tigerstedt, as *Trichosporium fuscum*, Herb. H; (31) Södermanland, Hyltinge parish, Henaredalen, 1.V.1937, Lundell & Ridelius, DAOM 49996; (32) Prov. Lublin, Nova-Alexandria, Poland, 3.IV.1910, Nevodovski, as *S. fusca*, Herb. WIS; (33) Prov. Vidzeme, Kr. Riga: Sigulda, Latvia, 12.V.1935, Smarods, as *S. fusca*, Herb. BPI. On CRATAEGUS: (34) [near London, Ont.], 14.IV.1890, Dearness, as *S. atra*, Herb. Dearness; (35) [near London, Ont.], 22.VI.1892, Dearness, as *S. atra*, Herb. Dearness; (36) Peters Woods [London, Ont.], March 1935, E.D., as *S. atra*, Herb. Dearness; (37) near Michigan Agric. College, Mich., Dec. 1891, Hicks, as *S. atra*, Herb. BPI. On FAGUS: (38) Port Stanley, Ont., 27.VI.1889, Dearness, as *Strumella ?coryneoidea*, Herb. Dearness; (39) Avon, Ont., 18.X.1897, Dearness, as *Streptothrix atra*, Herb. Dearness; (40) South of Aurora, Ont., 1.V.1932, Jackson, as *S.* sp., Herb. TRT; (41) Chalk River, Ont., 22.VI.1950, Fytche & Eggerton, as *S. fusca*,

Herb. TRT, (42) Hull, Que., 5.XI.1898, Macoun, as *S. atra*, DAOM 36865; (43) near Ithaca, N.Y., 6.IV.1904, Van Hook, as *S. atra*, Herb. IND; (44) Ithaca, N.Y., 6.IV.1904, Kauffman, as *S. atra*, Herb. MICH; (45) Ringwood Preserve, Ithaca, N.Y., 24.IV.1938, Solheim, as *S. atra*, Herb. Solheim; (46) Huckleberry Hill, Bloomington, Ind., 12.III.1910, Owens, as *S. atra*, Herb. IND; (47) Salt Creek, 6 miles south of Bloomington, Ind., 17.XII.1933, McCoy, as *S. atra*, Herb. IND; (48) Greencastle, Putnam Co., Ind., May 1909, Banker (1309), as *S. atra*, Herb. NY; (49) Kettle Creek, Potter Co., Pa., 28.V.1932, White, as *S. atra*, Herb. PAC; (50) Gray's Run, Lycoming Co., Pa., 20.IX.1925, Overholts, as *Strumella coryneoidea*, DAOM 38007; (51) Maysville, Armstrong Co., Pa., 26.XI.1932, White (1081), as *Streptothrix atra*, Herb. FH; (52) Mt. Lake Biol. Sta., Va., 3.IX.1936, Diehl, as *S. atra*, Herb. BPI; (53) Morgantown, W. Va., 2.IV.1936, Miller, Orton, & White (2375), as *S. atra*, Herb. FH. On FRAXINUS: (54) ?Preston, Ohio, 21.I.1887, Morgan, as *S. fusca*, Herb. IA. On HAMAMELIS: (55) Ann Arbor, Mich., 17.IV.1893, L.N. [Johnson] as *S. atra*, Herb. MICH. On JUGLANS: (56) Ann Arbor, Mich., 24.IV.1893, Johnson, as *S. atra*, Herb. NY. On NEMOPANTHUS: (57) W. Albany, N.Y., (undated), Peck, as *S. atra*, Herb. Dearness. On OSTRYA: (58) [near London, Ont.], 1.I.1892, Dearness, as *S.* sp., Herb. Dearness; (59) South Aurora, Ont., 15.VIII.1932, Jackson, as *S. fusca*, Herb. TRT; (60) South Aurora, Ont., 25.IX.1935, Cain, as *S. fusca*, Herb. TRT; (61) Devines Woods, Vaughns (Hudson Falls), Wash. Co., N.Y., 12.IV.1915, Burham (31), as *S. atra*, Herb. Dearness. On PRUNUS: (62) [London, Ont.], 3.VII.1904, Dearness, as *S. atra*, Herb. Dearness; (63) Turkey Creek, Johnson Co., Iowa, 7.V.1926, Adams & Martin, as *S. fusca*, Herb. BPI; (64) Madison, Wisc., 23.III.1916, Gardner, as *Strumella* sp., Herb. WSP. On QUERCUS: (65) New Fairfield, Conn., Aug. 1935, Battistce, as *Strumella coryneoidea*, Herb. BPI. On RIBES: (66) ?Preston, Ohio, 9.II.1891, Morgan, as *Streptothrix fusca*, Herb. IA. On TAXUS: (67) Palisades State Park, Iowa, 3.V.1947, Anderson, as *S. fusca*, Herb. IA. On TILIA: (68) Lake Geneva, Wisc., July 1903, E.T. & S.A. Harper, as *S. atra*, Herb. NY; (69) on ?*Tilia*, Michigan Hollow, Danby, N.Y., 21.X.1937, Solheim, as *S. atra*, Herb. FH. On ULMUS: (70) Madison, Dane Co., Wisc., 4.V.1945, Greene, as *S. fusca*, Herb. WIS. On UNIDENTIFIED BRANCHES: (71) [near ?Hull, Que.], 26.IX.1903, Macoun, as *S. atra*, DAOM 36866; (72) London, Ont., 24.IX.1903, Dearness, as *S. atra*, DAOM 36862; (73) Adelaide Woods [near London, Ont.], 8.IX.1928, Dearness, as *S. fusca*, Herb. Dearness; (74) London, Ont., 25.VI.1889, Dearness, as *Strumella coryneoidea*, Herb. NY; (75) Columbia, Mo., Spring 1945, Maneval, as *Streptothrix fusca*, Herb. UMO; (76) near Columbia, Mo., 28.X.1939, Routien, as *S. fusca*, Herb. FH; (77) Ohio, Morgan (517), as *S. fusca*, Herb. NY; (78) ?Preston, Ohio, Morgan, as *S. fusca*, Herb. IA; (79) Iowa City, Iowa, 21.VII.1956, Martin (8986), Herb. IA; (80) North Liberty, Iowa, 26.IV.1939, Martin, as *S. fusca*, Herb. BPI; (81) Iowa City, Iowa, 5.IV.1939, Emery, as *S. fusca*, Herb. IA; (82) Senfals Woods [between Iowa City and Cedar Rapids, Johnson Co.], Iowa, 10.XI.1908, Macbride, as *S. atra*, Herb. IA; (83) Fern.


A
B
B
A
C

Putnam Co., Ind., Nov. 1891, Underwood, as *S. atra*, Herb. NY; (84) Greencastle, Ind., 1892, Underwood, as *S. atra*, Herb. NY; (85) Michigan, Hicks (142) as *S. atra*, Herb. NY, (86) Central N.Y., Underwood, as *S. atra*, Herb. NY; (87) Fall Creek Woods, Cayuga Lake Basin, N.Y., 1.X.1905, Paul, as *S. atra*, Herb. CUP; (88) opposite Sycamore I., Fairfax Co., Va., 1904, Patterson, Charles & Dyre, as *S. atra*, DAOM 36868; (89) Flora of Fayette Co., W. Va., No. 1329, 16.XII.1893, Nuttall, as *S. atra*, Herb. MICH; (90) Univ. Wisconsin Arboretum, Dane Co., Wisc., 5.IX.1953, Cooke & Shaw, as *S.* sp., Herb. WSP.

CONOPLEA GLOBOSA (Schw.) Hughes, Can. J. Botany, 36: 755. 1958 (Fig. 4).
≡*Trichoderma globosum* Schw., Schrift. naturf. Ges. Leipzig, 1: 77. 1822; also in Fries, Syst. Mycol., 3: 215. 1832/33.
≡*Oidium inquinans* Schw., Trans. Amer. Phil. Soc., II, 4: 286. 1832.
≡*Torula inquinans* (Schw.) Sacc., Syll. Fung., 4: 251. 1886.
≡*Streptothrix globosa* (Schw.) Hughes, Can. J. Botany, 31: 606. 1953.
=*Cladosporium polysporum* Link, Linn. Spec. Plant., IV, 6 (1): 40. 1824.
=*Strumella coryneoidea* Sacc. & Wint. in Rabenh., Fungi Europ., No. 2984, anno 1883, and Hedwigia, 11: 175. 1883 (Nov.).

Mature fructifications are brown, dry and powdery, pulvinate, up to 3 mm in diameter, scattered or crowded and sometimes confluent.

The mycelium is immersed. Aggregations of fungal cells below the periderm eventually burst through the outer layers of the host to form pulvinate stromata over the surface of which densely crowded conidiophores are produced.

Conidiophores are arborescent, composed of a main stalk which is poorly differentiated and upwardly directed with often unilateral, primary, secondary, and tertiary branches. They are rough-walled along their whole length, hyaline to pale brown, more or less thin-walled and readily collapsing, irregularly flexuous, and up to 650 μ long and 4–6.7 μ wide. The bases of main stalks and some lower primary branches are cylindrical; other parts of the conidiophores are helically twisted. Sporogenous branchlets are borne laterally on the distal ends of conidiophores, usually one below a septum; they are up to 6 μ long and are denticulate with crowded conidium scars. The basal regions of conidiophore main stalks show frequent anastomoses. At maturity some of the conidiophore cells increase in length by intercalary growth and the component cells or lengths of the conidiophore become detached, one from the other, so that the fructification becomes composed of a dry powdery mass of conidia and fragmented conidiophores. Such elongated cells frequently show roughened rings alternating with smooth rings of cell wall indicating localized intercalary growth. Around the immediate base of the stromata, conidiophores are shorter and darker, more sparingly branched, somewhat thicker-walled not readily collapsing, and they bear but few conidia.

FIG. 4. *Conoplea globosa*: A, portions of two conidiophores, and conidia from collection No. 42 (p. 682); B, fragmented conidiophore cells, some with anastomoses, from collection No. 17 (p. 681); C, portions of conidiophores showing results of intercalary growth, with rough-walled rings alternating with smooth walls, from collection No. 58 (p. 682). ×1000

Conidia are terminus spores and are produced in large numbers in compact clusters on conidiophore branches and sporogenous branchlets particularly in the upper half or two thirds of the conidiophores. During development of the conidium, a thin hyaline separating cell may or may not be differentiated. They are oval, somewhat attenuated towards the base, sometimes subspherical or even angular in outline, with an inconspicuous basal scar, sometimes with a marginal frill, dark brown, thick-walled, coarsely roughened, with a narrow, hyaline, longitudinal (?)germination slit; they measure 6.7–8.1(–12.1) × 4.7–5.8 μ.

Hosts: *Acer*, *Betula*, *Carpinus*, *Carya*, *Castanea*, *Hicoria*, *Juglans*, *Quercus* (*alba*, *alba* var. *latifolia*, *borealis*, *garryana*, *macrocarpa*, *montana*, ? and other spp.).

Geographical sources of collections: Canada (Ont.), U.S.A. (N.Y., R.I., N.J., Conn., N.H., Mass., Pa., S.Car., La., Ala., Mo., Ohio, Iowa, Mich., Minn., Ore., Texas), Europe (Germany).

Collections Examined

Type and authenticated collections published exsiccati and some classical collections:

(1) The neotype collection of *Trichoderma globosum* Schw., '2751-8-Syn. Fung. *Oidium inquinans* Schw. in ramis *quercinis* &c. ex Herb. Schw. Beth[lehem, Pa.]. [scr. Michener]' in Herb. BPI. A probable duplicate of this collection is preserved in Herb. PH labelled by Michener '2751-8-Syn. Fung. *Oidium inquinans*—Schw. in ramos *Quercinos*. Beth'.

Schweinitz described *Trichoderma globosum* (≡*Oidium inquinans* Schw., 1832) in 1822 as follows, '*T.* magnum globosum pulvinatum confluens cinereum, demum olivaceo-nigrum. Copiose propullulat e ramis emortuis majoribus, praesertim *Liriodendri*, agmine saepe ulnari. —Fila per lentem vitream pellucida, hyalina, nigredine vix notabili afflata, septis raris vix conspicuis. Sporidia ovata, subpellucida, fusca'. Evidently the name *T. globosum* is polytypic but I have seen none of Schweinitz' collections from the type locality, North Carolina, in any N. American or European herbarium. In 1832 Schweinitz redescribed *T. globosum* as 'O[*idium*]. *inquinans*, L.v.S., Syn. Car. *Trichoderma globosum*, 524. Fungum hunc vulgatissimum, ulnari longitudine globosis pulvinulis, saepe confluentibus, occupantem ramos etiam *Quercinos*, *Oidium* esse non amplius dubitandum. Floccis erectiusculis articulatis simplicibus, demum solutis in Sporidia fuligineo-atra'. It is a collection on *Quercus* which is chosen as neotype, and, although it has suffered during storage, it shows sufficiently the essential characters to explain the name. At least one of the two collections next listed below is authenticated for *T. globosum* or *Oidium inquinans*.

(2) '*Oidium inquinans* L.v.S. Herb. Schw. ?*Streptothrix* Curtis [scr. Berk; on a corticated branch]' in Herb. K.

(3) '*Trichoderma globos.* [scr. ?] Schwaegrichen misit [scr. ?] Herb. E. Fries', in Herb. UPS. The association between the editor Schwaegrichen and Schweinitz has been mentioned by Rogers (1944, p. 528). It is quite possible

that this collection was sent by Schweinitz to Schwaegrichen, who then sent it to Fries. It was indicated by Fries (1832/33, p. 215) that he had seen a collection of *T. globosum* from Schweinitz and this is very probably the one. This suggestion is supported by the fact that another label in Herb. Fries with '*Dematium fuligo*' written on it in the same hand as '*Trichoderma globos.*' also has 'Schweinitz' added. Fries (1832/33, p. 379) also indicated that he had seen a specimen of *D. fuligo* from Schweinitz.

(4) The presumed type collection of *Cladosporium polysporum* Link (Fig. 20), '*Cladosporium polysporum*, Berol. [scr. Link]', in Herb. B. Link described his species on rotten wood from Berlin; the only specimen assigned to *C. polysporum* in Herb. B. is from Berlin but is on bark. This is the only collection of *Conoplea globosa* known to me from Europe.

(5) The type collection of *Strumella coryneoidea* Sacc. & Wint., 'Rabenhorst-Winter, Fungi Europaei 2984. *Strumella coryneoidea* Sacc. et Winter nov. spec. . . America borealis; Perryville, Missouri ad *Quercus albae* L. ramos aridos. April 1883, leg. C.H. Demetrio', in Herb. BPI, CUP, FH, and NY. The four specimens of this type collection that I have seen are all in poor condition but show enough to indicate the identity of the fungus. Collection No. 28 listed below is on the type host and from the type locality.

(6) Ellis, North Amer. Fungi, 131. *Streptothrix atra*, B. & C., on dead oak limbs. Herb. NY.

(7) Ellis & Everh., North Amer. Fungi (Ser. 2), 1653, *Strumella coryneoidea* Sacc. & Wint., on bark of oak limbs, Perryville, Mo., C. H. Demetrio, June 1885. Herb. CUP.

(8) Ellis & Everh., Fungi Columbiani, 454. *Streptothrix atra*, B. & C., on *Carpinus americana*, London, Ont., May 1893, Dearness. Herb. NY.

(9) Thümen, Mycotheca Universalis, 86. *Streptothrix atra* Berk. & Curt., on *Quercus*, Newfield, N.J., J. B. Ellis, May 1874. Herb. NY.

(10) Reliquiae Farlowianae, 202*b*. *Streptothrix atra*, on *Quercus*, Sharon, Mass., Oct. 1911, A. P. D. Piguet. DAOM 29010. No. 202*a* in this series bears *Conoplea sphaerica*.

(11) 'Ravenel. Fungi Caroliniani Exsiccati. Fasc. 3. 83. *Streptothrix atra*, Berk. et Curt.! ramulis mortuis *Quercus*'. Herb. NY.

(12) 'No. 3581. *Streptothrix atra* in *Carpinum americ.* Pennsylv. [scr. Berk.]', in Herb. K. This is one of the cotype collections of *S. atra* (cf. p. 689).

(13) '*Streptothrix atra*, Berk. & Curt. 1605. Santee River [S. Car.; on a twig; scr. Berk.]', in Herb. K. This collection was mentioned as an unnamed variety of *S. atra* by Berkeley (cf. p. 689).

(14) '*Streptothrix atra*, B. & C. *Querc*: ram. mort. S. *Carolina*—hieme. M.A. Curtis [scr. presumably Curtis] Herb. E. Fries', in Herb. UPS.

Other collections: on ACER: (15) N.H., Blake (121) as *S. atra*, Herb. NY. On BETULA: (16) Sharon, Mass., Oct. 1890, Galloway, as *S. ?fusca*, Herb. NY. On CARPINUS: (17) Natchaug State Forest, Eastford, Conn., 13.I.1934, Bidwell & Moses, as *Strumella coryneoidea*, Herb. PAC. On CARYA: (18) Perryville, Mo., 2.IV.1915, Overholts (2729) & Studhalter, as *S. coryneoidea*.

Herb. BPI. On CASTANEA: (19) State College, Pa., 25.X.1918, Overholts, as *S. coryneoidea*, DAOM 2337; (20, 21) St. Mary's, Pa., 13.III.1913, Heald (229, 230), as *S. coryneoidea*, Herb. NY; (22) Reitz Gap, Center Co., Pa., Feb. 1934, Aaron, as *S. coryneoidea*, DAOM 3546. On HICORIA: (23) Detwiler Run, Center Co., Pa., 20.XI.1922, Overholts, as *S. coryneoidea*, Herb. PAC; (24) Diamond Valley, Hunt Co., Pa., 21.XI.1925, Overholts (10109), as *S. coryneoidea*, DAOM 2336. On JUGLANS: (25) State College, Pa., 6.VIII.1949, Fergus, as *S. coryneoidea*, Herb. PAC. On QUERCUS ALBA: (26) State College, Pa., 7.V.1933, Bidwell & White (1124), as *S. coryneoidea*, Herb. NY; (27) State College, Pa., 11.VI.1920, Orton & Overholts, as *S. coryneoidea*, Herb. NY; (28) Perryville, Mo., 2.IV.1915, Studhalter & Overholts (2730), as *S. coryneoidea*, Herb. NY; (29) Killingworth, Conn., 10.IX.1935, Bidwell, as *S.* sp., Herb. BPI. On QUERCUS ALBA var. LATIFOLIA: (30) Salamanca, N.Y., 11.X.1931, Hirst & Harris, as *Streptothrix atra*, Herb. BPI; (31) Salamanca, N.Y., 22.II.1932, Harris, as *Strumella coryneoidea*, Herb. Dearness. On QUERCUS BOREALIS: (32) Campton, N.H., 25.IX.1934, Hansbrough, as *S. coryneoidea*, Herb. NY; (33) Housatonic State Forest, near Cornwall, Conn., 26.X.1933, Welch, as *S. coryneoidea*, Herb. BPI; (34) Walpole, Mass., 16.III.1933, Hansbrough (162) as *S. coryneoidea*, Herb. NY. On QUERCUS GARRYANA: (35) Benton Co., Ore., Oct. 1923, Zeller, as *S. coryneoidea*, Herb. NY. On QUERCUS MACROCARPA: (36) State College, Pa., 23.V.1949, Fergus, as *S. coryneoidea*, Herb. PAC; (37) State College, Pa., 24.VI.1957, Newman, DAOM 63544. On QUERCUS MONTANA: (38) Logan State Forest, Pa., 18.X.1944, Marshall & Spaulding, as *S. coryneoidea*, Herb. Solheim. On QUERCUS: (39) [near London, Ont.], 7.IX.1896, Dearness, as *Streptothrix atra*, Herb. Dearness; (40) London, Ont., 7.IX.1896, Dearness, as *S. atra*, DAOM 36861; (41) Enfield Ravine, Cayuga Lake Basin, N.Y., 19.X.1894, Nichols, as *S. atra*, Herb. CUP; (42) Newfield, N.J., Dec. 1879, Ellis (1387), as *S. atra*, Herb. NY; (43) East Providence, Rhode I., Oct. 1896, as *S. ?atra*, Herb. FH; (44) Bedford, Mass., Aug. 1901, as *S.* sp., Herb. FH; (45) Huntingdon Res., Pa., 8.X.1913, Heald & Studhalter (226), as *Strumella coryneoidea*, Herb. NY; (46) Charter Oak, Pa., 23.XI.1927, Spaulding & Overholts, as *S. coryneoidea*, Herb. BPI; (47) Ross Run, Hunt Co., Pa., 1.III.1931, Mounce, Overholts (13082), & Moses, as *S. coryneoidea*, DAOM 2335; (48) Ross Run, Pa., 1.III.1931, Overholts & Mounce, as *S. coryneoidea*, DAOM 20057; (49) near Ann Arbor, Mich., 2.III.1895, Schaffner & Peters, as *S. coryneoidea*, Herb. MICH; (50) near Northfield, Minn., Fall of 1933, Bramble, as *S.* sp. Herb. BPI; (51) Perryville, Mo., 2.IV.1915, Overholts, as *S. coryneoidea*, Herb. Dearness; (52) Houston, Tex., 19.IV.1869, H.W.R. (301), as *Streptothrix atra*, Herb. BPI. On UNIDENTIFIED BRANCHES; (53) Harraby, Lake Rosseau, Ont., Sept. 1902, E. T. & S. A. Harper, as *S. atra* (not on pine sticks as stated), Herb. NY; (54) Westville, Conn., 28.VIII.1901, Rorer, as *S. atra*, Herb. BPI; (55) ?Preston, Ohio, Morgan, as *S. abietina*, Herb. IA; (56) Iowa, Sept. 1889, Macbride, as *S. atra*, Herb. IA; (57) Columbia, S. Car., Fall of 1888, Atkinson, as *S. abietina*, Herb. CUP; (58) Abita Springs, East

La., 1.IV.1898, Langlois, as *S. atra*, Herb. NY; (59) Auburn, Lee Co., Ala., Nov. 1895, Underwood, as *S. atra*, Herb. NY; (60) Auburn, Ala., Jan. 1896, Earle & Underwood, as *S. atra*, Herb. NY; (61) Houston, Tex., 10.IV.1869, H.W.R., as *S. atra*, Herb. NY.

In 1914 Heald and Studhalter published an account of 'The *Strumella* [*coryneoidea*] disease of Oak and Chestnut trees'. It is evident from their description and illustrations that they had *Conoplea globosa* under consideration.

CONOPLEA FUSCA Pers., Mycol. Europ., 1: 12. 1822 (Fig. 5).
≡*Oidium subramosum* Link, Linn. Spec. Plant., IV, 6 (1): 125. 1824.
≡*Oospora subramosa* (Link) Wallr., Flora Crypt. German., 2: 183. 1833.
≡*Torula subramosa* (Link) Sacc., Syll. Fung., 4: 249. 1886.
≡*Dematium fuscum* (Pers.) Sacc., Syll. Fung., 10: 591. 1892.
=*Streptothrix fusca* Corda, Pracht-Flora. . . p. 27. 1839.
≡*Monosporium curvatum* Bon., Handbuch. . .Mykol., p. 96. Stuttgart. 1851.
=*Acremonium vaccinii* Fuckel, Symb. Mycol., p. 362. 1870.
≡*Acremoniella vaccinii* (Fuckel) Sacc., Syll. Fung., 4: 303. 1886.
=*Trichosporum crispulum* Sacc. & Malbr. in Sacc., Michelia, 2: 640. 1882, as *Trichosporium*.
≡*Colletosporium crispulum* (Sacc. & Malbr.) O. Kuntze, Rev. Gen. Plant., 2: 849. 1891.
=*Trichosporum tortuosum* Karst., Meddel. Soc. F. F. Fennica, 14: 90. 1887, as *Trichosporium*.
=*Botrytis torta* Ell. & Everh., Proc. Acad. Nat. Sci. Phil., 1894: 375. 1894.
=*Streptothrix mounceae* Sumst., Mycologia, 29: 250. 1937.

Conoplea fusca forms brown, dry, and powdery fructifications; these may be separate and pulvinate or so close together that they form extensive unbroken colonies on the substratum.

The mycelium is immersed but on stems of *Eupatorium*, at least, it does not penetrate deeply into the host tissues; the hyphae are subhyaline to brown, branched, septate, smooth, and 1.5–4.5 μ wide. At intervals, aggregations of cells form small, flattened or raised, superficial or partly immersed stromata from which rooting hyphae radiate.

Conidiophores are crowded on the stromata, are erect in the center, but towards the margin they become more and more divergent. They are septate, pale brown to brown, helically twisted, composed of an usually straight or slightly bent main stalk bearing at different levels, but usually in the lower half, one to five generally alternate, sometimes opposite lateral primary branches which are directed upwards, up to 250 μ long, up to 3.8 μ wide, and which may themselves occasionally bear secondary branches; they are rough-walled except at the immediate base. Main stalks are up to 400 μ long and 4 μ wide, and these and the lateral branches finally terminate in sporogenous cells. Sporogenous branchlets are produced laterally, usually one below each of the upper septa of the main stalk and its branches; these are straight or curved,

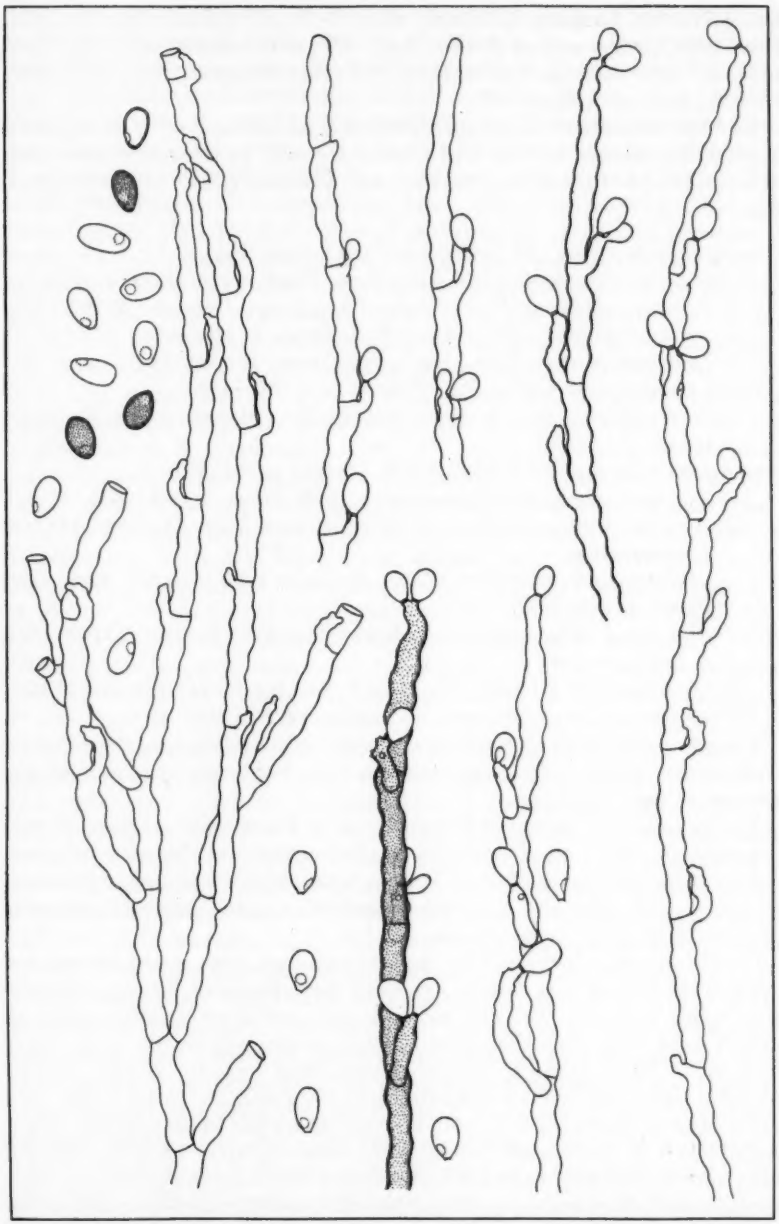


FIG. 5. *Conoplea fusca*, conidiophores and conidia from the type collection of *Streptothrix mounceae* (DAOM 38427). ×1000

subcylindrical, subhyaline to pale brown, divergent or adpressed to the conidiophore, irregular in outline with conidium scars, and up to 37 μ long and 2–2.5 μ wide.

Conidia are terminus spores and are produced on the sporogenous branchlets and on the fertile ends of the main stalk and its branches. During development of the conidium, a thin (0.8 μ), hyaline separating cell is differentiated. They are oval to obovate, narrowed very slightly towards the base, with an inconspicuous basal scar, usually with an inconspicuous marginal frill, pale brown to brown, roughened most inconspicuously, with a circular, paler, and thinner area of wall (?germination pore) about 2 μ in diameter usually just above the basal scar; they measure 5.8–8.1(–12)×4–5.2(–6.4) μ.

Hosts: *Carex*, *Eupatorium*, *Myrtillus*, *Populus*, *Pseudotsuga*, *Rosa*, *Rubus*, *Vaccinium*.

Geographical sources of collections: Canada (Ont., Sask., B.C.), U.S.A. (Colo., N.J., W.Va.), Europe (Finland, France, Germany).

Collections Examined

Type collections:

(1) The type collection of *Conoplea fusca* Pers. (Fig. 16), '*Conoplea fusca* [scr. Pers.] *Helmisporium subramosum* m. in caulib. ruborum lecti. Lipsiae: forsan ad g[enus]. *Acladium* pertinet, nam sporidia sphaerica [scr. Kunze] Hb. Pers. ded. Kunze [scr. J.H.S.S.]', in Herb. L (910. 263–438).

Persoon followed his brief diagnosis of *Conoplea fusca* with the statement, 'Circa Lipsiam a D. Kunze lecta, mecumque sub nomine *Helmisporium subramosum* communicata' so there is no doubt about the identity of the type collection. It appears that Kunze sent a part of his collection to Link also, and this part is preserved in Link's herbarium in Herb. B (Fig. 15). It is labelled as follows: '*Oidium subramosum* [scr. Link] *Helmisporium subramosum* m. [scr. Kunze.] *Cladosporium pulvinatum* m. [scr. Link; nomen nudum]. *H*[*elmisporium*]. *minus* Tibi floccis sparsis, [*Helmisporium*] *ramosissimum* Tibi, floccis ramosissimis, sporidiis ad basin adhaerentibus differe rident. Lips. in caulibus Ruborum lecti, aestate [scr. Kunze]'.

Link (1824) cited *Conoplea fusca* Pers. as a synonym of *Oidium subramosum* Link. He added 'Habitat in ramis Rosarum [*sic*] dejectis in Germania, Lect. circa Lipsiam (Kunze). (v.s.)'. Although Link gave the host as *Rosa*, and not *Rubus* as indicated on the label by Kunze, I am convinced that both Persoon and Link examined parts of the same collection.

(2) The type collection of *Acremonium vaccinii* Fuckel, Fuckel's Fungi Rhenani 158, 'Ad *Vaccinii vitis-idaeae* ramulos aridos adhuc gerentes, rarissime Autumno. In sylva Hostrichiensi', in Herb. G.

A distribution ex 'Herbier Barbey-Boissier' of Fuckel's material, under the number 2552 and the name *Acremoniella vaccinii* (Fuckel) Sacc. on *Vaccinium vitis-idaeae*, is very probably part of this collection.

(3, 4) The cotype collections of *Trichosporum crispulum* Sacc. & Malbr. viz. Libert 998 on *Rosa*, and Malbranche, on *Vaccinium myrtilli*, Brionne. (3)

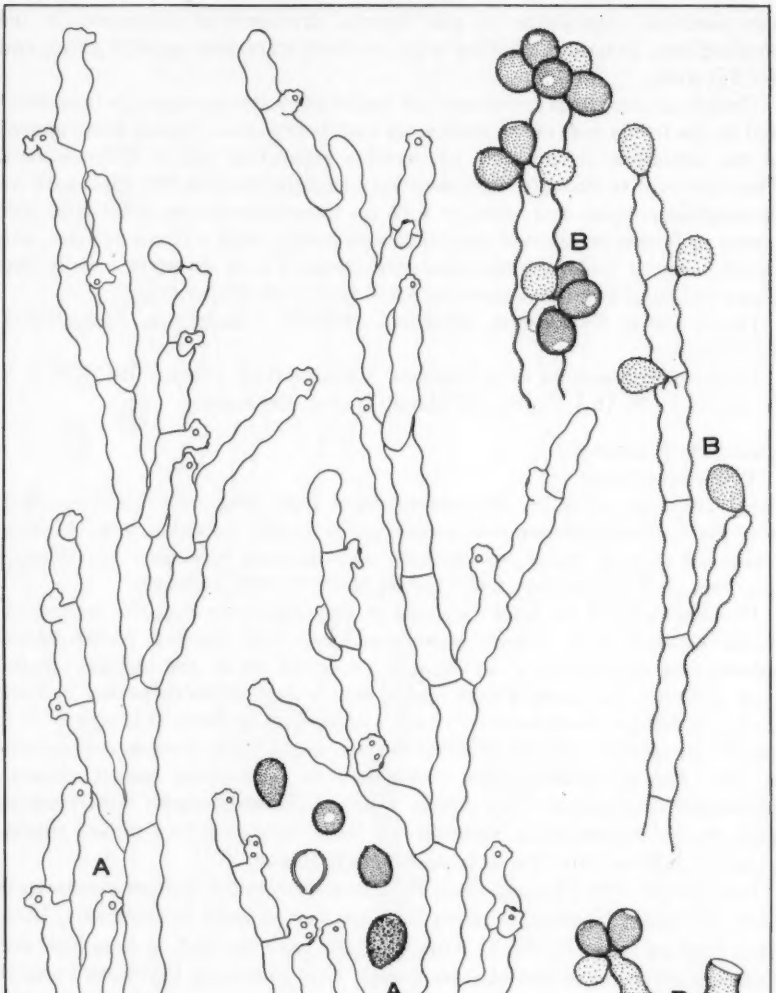


FIG. 6. *Conoplea juniperi* var. *juniperi*, conidiophores, and conidia: A, from collection No. 7 (p. 689); B, from collection No. 3 (p. 689). ×1000

'*Trichosporium crispulum* Sacc. & Malbr. in ramulis *Myrtilli*. Brionne. Malbranche. 8.80 [scr. Sacc.]', in Herb. PAD. (4A) '*Trichosporium crispulum* Sacc. & Malbr. [scr. Sacc.] 998. *Sporotrichum elaeochroum* Fr. var. les sporidies ovales non [. . .] sur tiges de Rôsiers. Lb [Libert] [scr. ?]', in Herb. PAD. (4B) A part of the same collection is preserved in Herb. K, with the following label: '*Trichosporium crispulum* Sacc. [scr. Cooke] *Sporotrichum elaeochroum* Fr.[5] ad ramos rosarum et ruborum etc. [on *Rosa*]; obs. les sporidies sont plus ovales que rondes. Coll. Libert — No. suppl. 998'. (4C) Roumeguère's Fungi Gallici Exsiccati 1769. '*Trichosporium crispulum* Sacc. & Malbranche. Branches pourrissantes des Rosiers. Malmedy (Reliquiae Libertianae)', in Herb DAOM (36164), is probably a distribution of Libert 998.

(5) The type collection of *Trichosporum tortuosum* Karst., '*Trichosporium tortuosum*, Mustiala ad *Caric. digit.* ? *palulifer.* 8 Apr. 1866 [scr. Karst.]', in Herb. H.

(6) The type collection of *Botrytis torta* Ell. & Everh., 'No. 257 [scr. ?] *Botrytis torta* E. & E. [scr. Ellis] on a rare plant—*Carex frazeri* [scr. presumably Nuttall]. Flora of Fayette Co., W.Va. Collected by L. W. Nuttall'. in Herb. NY.

(7) The type collection of *Streptothrix mounceae* Sumstine, [on bark of dead twig] 'Kenora, Ont., Sept. 25. 1932. G. R. Bisby', Herb. DAOM 38427.

Other collections: (8) '*Streptothrix*, on stem of EUPATORIUM: Newfield [N.J.], Oct. 25. '79 [scr. Ellis]', Herb. NY. On POPULUS: (9) Candle Lake, Sask., 18.VII.1957, Zalasky, DAOM 59604. On PSEUDOTSUGA: (10) F.E. & E.S. Clements, Crypt. Formationum Coloradensium, 269, Minnehaha, 5.VII.1906, as *S. atra*, Herb. NY and CUP. On RUBUS: (11) '*Sporotrichum torulosum* Mspt. [nomen nudum] Leipzig, an faulenden *Rubus* Ranken, Auerswald [scr. Auersw.]', Herb. Fuckel in Herb. G. On VACCINIUM VITIS-IDAEAE: (12) '*Trichosporium crispulum* Sacc., Vasa [Finland] in *Vacc. vit.-id.*, 20 Apr. 1865 [scr. Karsten]', Herb. H. On UNIDENTIFIED WOOD: (13) Courtenay, Vancouver I., B.C., 21.VIII.1957, Hughes, DAOM 56334.

In collections 9, 10, and 13, the germination pore is about half way along the length of the conidium and not predominantly just above the scar as in the other collections. At present I feel that this difference is insufficient to merit the proposal of a new taxon.

CONOPLEA JUNIPERI Hughes var. *juniperi*, Can. J. Botany, 36: 755. 1959 (Fig. 6).

≡*Streptothrix atra* Berk. & Curt. in Berk., Grevillea, 3: 107. 1875, non *Conoplea atra* Pers.

C. juniperi forms reddish-brown, dry, and powdery fructifications on the bark and wood of *Juniperus virginiana*; these are pulvinate, 200–700 μ in diameter when solitary but by confluence they may form extensive patches up to 3 cm long.

[5]Professor J. A. Nannfeldt kindly informed me (in litt., 4.X.1954) that there is no material of *Sporotrichum elaeochroum* Fr. preserved in the Friesian Herbarium at Uppsala.

The mycelium is immersed and composed of 2–4.5 μ wide hyphae which do not penetrate deeply into the bark; the innermost hyphae are hyaline and the outer ones dark brown. The outer hyphae are short-celled and irregular, forming knots within the cork cells and here and there these knots proliferate, burst through the cork cells, and form superficial, compact, dark-brown, subglobose stromata which are up to 100 μ in diameter.

Conidiophores are densely crowded on the stromata, are erect in the center, but towards the margin they radiate more and more outwards. They are septate, pale brown to dark brown, rough-walled, and helically twisted except towards the base. They are composed of a straight or flexuous main stalk bearing alternate, rarely opposite, lateral primary branches which usually bear secondary branches; the main stalk is up to 500 μ long 4–5.4 μ wide below and 3–5 μ wide above, and this and the lateral branches finally terminate in sporogenous cells. Sporogenous branchlets are produced laterally, one below each of the upper septa of the main stalk and its branches; these are straight or curved, subspherical to oval, pale brown to brown, divergent, denticulate with conidium scars, and 3–10 μ long.

Conidia are terminus spores and are produced on the sporogenous branchlets and on the fertile ends of the main stalk and its branches. During development of the conidium a thin (0.5–0.8 μ), hyaline to subhyaline separating cell is differentiated. They are subglobose to oval, sometimes narrowed very slightly towards the base, with an inconspicuous basal scar, sometimes with a minute marginal frill, brown to dark brown, thick-walled, minutely and inconspicuously or coarsely roughened, with a circular paler, thin-walled area (?germination pore) 1.2–2 μ in diameter, usually at the apex but sometimes subapical; they measure (5.2–)6.0–7.5(–8.6)×4.7–5.8(–6.8) μ.

Host: *Juniperus virginiana*

Geographical sources of collections: U.S.A. (N.Y., N.J., Pa., Conn., Mass., Del., Md., N.Car., Ala., Ohio, Mich., Mo.).

Collections Examined

Type and authenticated collections, and published exsiccati:

(1) The lectotype collection of *Streptothrix atra* Berk. & Curt. (≡*Conoplea juniperi* Hughes), 'No. 4895. *Streptothrix atra*, B. & C. in *Junipero*. Massach[usetts]. C. J. Sprague [scr. Berk.]', in Herb. K.

Streptothrix atra was described as follows: 'Floccis parce articulatis sursum ramosis crenulato-flexuosis; sporis globosis vel subellipticis inquinantibus. . . . . Threads branched above, repeatedly undulated, with short constrictions; spores globose or subelliptic, abundant, falling off as a black powder. No. 1605, Santee River,[6] is a variety, with the threads far less undulated and the spores obovate'. Six collections were cited:

'On Juniper, Alabama, Peters. No. 4594'.

'Massachusetts, C. J. Sprague. No. 4895'.

'On *Carya*. Pennsylvania, Michener. No. 3827'.

[6]This is *Conoplea globosa* (q.v.)

'On *Carpinus americana*. Pennsylvania. No. 3581'.
'On Peach. Car. Inf. No. 2789'.
[On Peach. Car. Inf. No.] '3282'.

I have seen five of these six cotype collections in Herb. K. Numbers '4594' and '4895' are both on *Juniperus* and bear the same fungus; '4895' is designated the lectotype collection of *Streptothrix atra* Berk. & Curt. Numbers '3827' on *Carya* and '3581' on *Carpinus* are *Conoplea sphaerica* and *C. globosa* respectively. Number '3282' on Peach is *Virgariella fuscopurpurea* (Berk. & Curt.) Hughes. I was unable to find '2789' in the *Streptothrix atra* folder in Herb. K; however, on the packet of '3282', Berkeley wrote 'No. 3282=2789', which accounts perhaps for the absence of '2789'.

'3282' would be unsuitable as lectotype because it does not produce 'Floccis.. ramosis crenulato-flexuosis'. '4895' is chosen because it matches the diagnosis better than either '3827' (*Conoplea sphaerica*) or '3581' (*C. globosa*). Furthermore, so far as I am aware, the taxon typified by '4895' has no other name available.

(2) '4594. *Streptothrix atra*, B. & C. in *Juniperus*. Alabama. Peters. [scr. Berk.]', in Herb. K, (vide supra).

(3) Ellis & Everh., North Amer. Fungi, 2686, *Streptothrix abietina* Peck, on *Juniperus virginiana*, Newfield, N.J., June 1889. Herb. NY.

(4) Shear, New York Fungi, 192, *Streptothrix atra*, on *Juniperus virginiana*, Alcove, N.Y., March 1893. C.L.S. Herb. NY.

Other collections: all on JUNIPERUS VIRGINIANA: (5) Vicinity of Cambridge, Mass., Farlow, as *S. atra*, Herb. NY; (6) Newton, Mass., Aug. 1892, Farlow, as *S. atra*, Herb. NY; (7) Newton, Mass., Dec. 1892, Farlow, as *S. atra*, Herb. NY; (8) Newton, Mass., Oct. 1892, as *S. atra*, Herb. FH; (9) Middlesex Fells, Mass., 28.X.1894, as *S. abietina*, Herb. MICH; (10) Sharon, Mass., Nov. 1909, Piguet, as *S. atra*, Herb. BPI; (11) Arlington, Mass., 23.II.1894, Schrenk, as *S. atra*, Herb. CUP; (12) Waverly, Mass., Apr. 1887, Miyabe, as *S. atra*, Herb. UC (49570); (13) Newton, Mass., 23.X.1887, Setchell, as *S. atra*, Herb. UC (49571); (14) Buffalo, N.Y., Clinton, as *S. atra*, Herb. NY; (15) Long I., N.Y., Zabriski, as *S. abietina*, Herb. NY; (16) Alcove, N.Y., May 1893, Shear, as *S. atra*, Herb. BPI; (17) Orient Point, Long I., N.Y., 14.XI.1910, 8.V.1911, Latham, as *S. fusca*, Herb. TRT; (18) Wilmington, Del., 2.I.1890, Commons, as *S. atra*, (stated to be on *Vaccinium corymbosum* but Mr. J. D. Hale, in litt. to me 22.I.1954, kindly identified the wood as *Juniperus* sp.), Herb. NY; (19) New Brunswick, N.J., 10.IV.1892, Stevens, as *S. atra*, Herb. BPI; (20) Cobalt, Conn., 16.I.1936, Eno, as *S. abietina*, Herb. IA; (21) Milford, Conn., 16.IV.1887, Setchell, as *S. atra*, Herb. UC (49569); (22) Norwich, Conn., 25.XI.1887, Setchell, as *S. ?atra*, Herb. UC (49568); (23) Norwich, Conn., 6.VII.1888, Setchell, as *S. atra*, Herb. UC (49572); (24) State College, Pa., 7.XII.1918, Overholts, as *Strumella* sp., Herb. BPI, PAC; (25) Cabin Hill, Plummer's I. in the Potomac River, near Cabin John, Montgomery Co., Md., 3.XI.1940, Leonard, as *Streptothrix atra*, Herb. BPI; (26) Plummer's I., Montgomery Co., Md., 5.XI.1944, Leonard, as *S. fusca*, Herb. BPI; (27) Bladensburg, Md., 24.XII.1940, Leonard, as *S. fusca*, Herb.

BPI; (28) Durham, N.Car., Apr. 1946 Linder, as *S. atra*, Herb. FH; (29) Duke Forest, N.Car., Spring 1936, Wolf, as *S.* sp., Herb. BPI; (30) Beaver Pond, Adams Co., Ohio, 15.X.1938, Manley, as *S. abietina*, Herb. FH; (31) Perryville, Mo., 2.IV.1915, Overholts, as *S.* sp., Herb. Dearness; (32) Cedar Bend, Ann Arbor, Mich., 8.IV.1893, L.N.J., as *S. atra*, Herb. MICH; (33) [no locality] '*Streptothrix atra*, on *Juniperus virginiana*. Jan. 1876 [scr. ?]', Herb. K.

CONOPLEA JUNIPERI var. **robusta** var. nov.

A typo ita differt: conidiophora usque ad 1100 μ longa; conidia majores, variabiles, 7–13.5×5.6–9.5 μ.

Typus, in foliis ramulisque *Abietis grandis*, Elk River, Idaho, 29.VII.1931, J. R. Hansbrough, 40710, in Herb. Dearness in Herb. DAOM.

Other collections: On ABIES GRANDIS: on dead leaves, Laird Park, Latah Co., Idaho, 25.V.1945, Shaw, Herb. WSP (35364); on leaves, leaf scars, and twigs, Bear Cr. Camp, Umatilla National Forest, N. Garfield Co., Wash., 7.VI.1949, Shaw, Yerkes, & Turley, Herb. WSP (21527 and 21554); on leaves and twigs, Aladdin, Wash., 3.VIII.1929, Hedgcock, as '*Trichosporium tubercularioides* [nomen nudum]', Herb. Dearness. On ABIES LASIOCARPA: dead leaves and twigs, Ione, Wash., 6.VII.1929, Hedgcock, as '*Trichosporium tubercularioides* [nomen nudum]', Herb. Dearness. On UNIDENTIFIED BRANCH: 'Washington's Flora, *Streptothrix* sp., Skamania Co., Gifford Peak, Chiquash Mts., Wilhelm N. Suksdorf, 521, Aug. 1895', Herb. WSP (28280).

Conoplea juniperi var. *robusta* forms reddish-brown fructifications up to 2 mm in diameter on dead leaves, leaf scars, and small twigs. In a specimen on bare unidentified wood, fructifications are up to 4 mm wide.

The mycelium is immersed, hyaline within the host, but becoming brown towards the outside. Brown stromata formed on leaves, leaf scars, and twigs are up to 100 μ in diameter and may be as small as 30 μ. On wood, definite stromata are not evident and the mycelium forms a dark layer just below the surface.

Conidiophores are densely crowded on the stromata and closely resemble those of *C. juniperi* except that the main stalk may be up to 1100 μ long and the lateral branches more distantly arranged. The sporogenous branchlets which are fertile along their whole length are up to 40 μ long and are conspicuously denticulate with conidium scars and may be geniculate or curiously bent.

Conidia are similar to those of *C. juniperi* but are more coarsely roughened with an apical or subapical thin-walled area (?germination pore). They show a greater range in size and are larger than those of *C. juniperi*, measuring 7–13.5×5.6–9.5 μ.

Hosts: *Abies grandis*, *A. lasiocarpa*, and an unidentified branch.

Geographical sources of collections: U.S.A. (Wash., Idaho).

On the packet of his collection, Hansbrough had written 'Saprophytic and mildly parasitic in lower needles on small trees. Common'.

CONOPLEA GENICULATA (Corda) Hughes, Can. J. Botany, 36: 755. 1958 (Fig. 7).

≡*Botrytis geniculata* Corda, Pracht-Flora . . . . p. 35. 1839.

≡*Monosporium geniculatum* (Corda) Bon., Handbuch . . . Mykol., p. 96. Stuttgart. 1851.

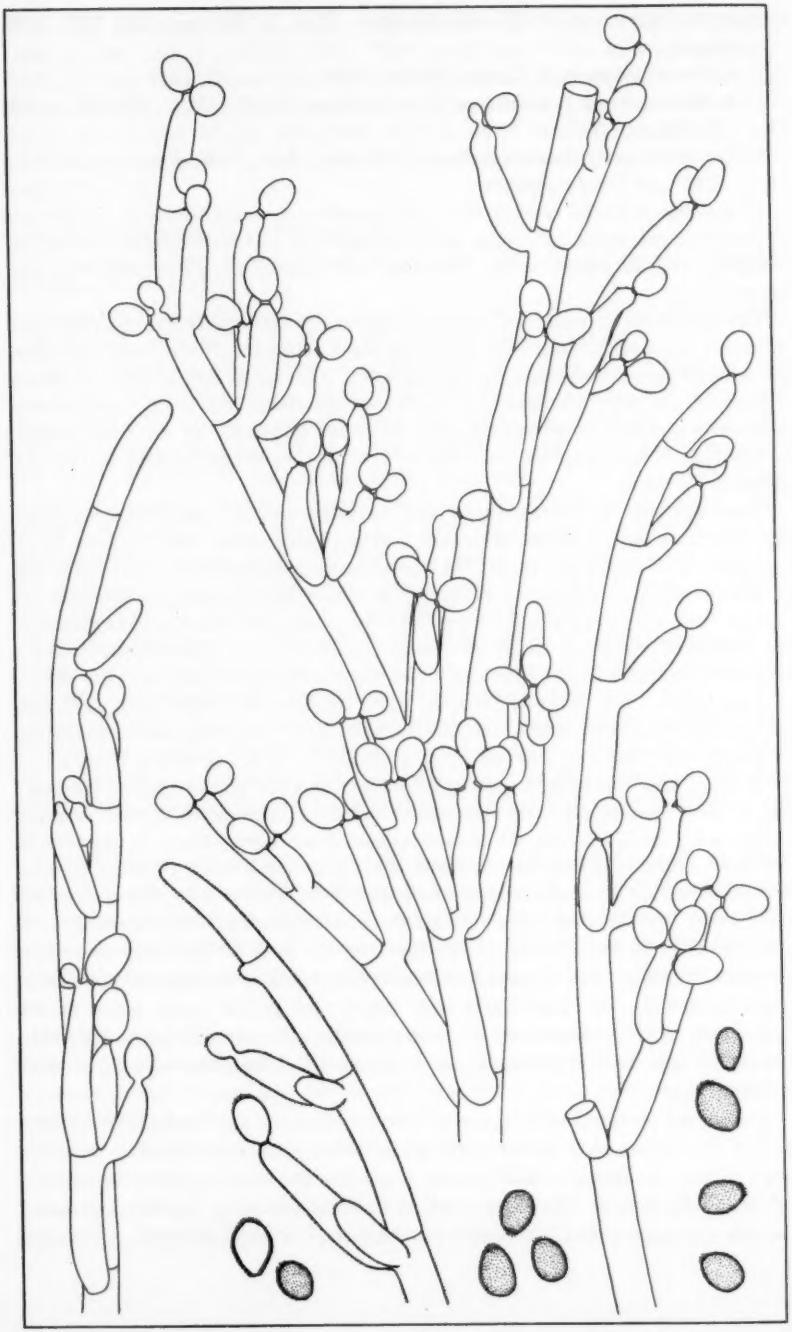
=*Trichosporum substrictum* Karst., Meddel. Soc. F. F. Fennica, 14: 107. 1887, as *Trichosporium*.

C. geniculata forms brown, dry, and powdery fructifications on corticated or decorticated branches; these may be separate and pulvinate or formed in irregular and extensive mats. With age the fructifications become blackish brown.

The mycelium in some collections is immersed and composed of subhyaline to brown hyphae, 1.5–6 μ wide, forming black stromata at intervals and these are subglobose, erumpent through the bark, and up to 400 μ wide. In other collections the mycelium may form a black rind about 25 μ thick, composed of dark-brown cells formed superficially and also immersed in the host tissues; in addition, repent hyphae may also be present and these are dark brown and about 6.5 μ wide.

Conidiophores are arborescent and crowded on the pulvinate stromata but less crowded on effuse stromata and scattered when they develop from the superficial repent hyphae. On the pulvinate stromata in particular the conidiophores which develop centrally are erect but proceeding towards the margin they are outwardly divergent; on the effuse stromata the conidiophores are produced either in small groups or in the form of discontinuous mats. The conidiophores from pulvinate stromata are regularly septate, pale brown to dark brown, not helically twisted, paler towards the distal ends, and conspicuously roughened except towards the base. They are composed of a straight or gently and regularly curved main stalk which is up to 900 μ long, up to 8.5 μ wide near the base, tapering to about 4 μ wide at the apex. This bears one or two, sometimes three, generally unilateral, upwardly curved primary branches up to 275 μ long which are longest when borne towards the base of the main stalk and shortest towards the apex; these may themselves bear unilateral, upwardly directed secondary branches. At maturity, the ends of the main stalk and its branches terminate in subcylindrical sporogenous cells which are up to 50 μ long, tapered somewhat towards the apex which is irregular in outline with conidium scars. Sporogenous branchlets are produced laterally, usually one, sometimes two, below each of the upper septa of the main stalk and its branches; these are straight or curved, long-oval, subhyaline to pale brown, divergent, and, at the apex, irregular in outline with conidium scars.

Conidia are terminus spores, and are produced on the sporogenous branchlets and on the fertile ends of the main stalk and its branches. During development of the conidium a thin (about 1 μ), hyaline to subhyaline separating cell is differentiated. They are oval and sometimes very slightly narrowed towards the apex, with an inconspicuous basal scar, sometimes with a marginal



frill, dark brown, thick-walled, roughened with short subhyaline spines, with a slightly paler, thin-walled circular area (?germination pore) at the apex, on which the spines are longer and conspicuous; the conidia measure 5.1–12.1×5–6.7 μ.

Hosts: *Abies balsamea*, *Corylus*, *Ledum palustre*, *Pinus* (*contorta*, *sylvestris*), *Populus*, *Vaccinium myrtillus*.

Geographical sources of collections: Canada (Nfld, Ont., Alta), U.S.A. (N.Y.), Europe (Finland, Czechoslovakia).

Collections Examined

Type and authenticated collections:

(1) The lectotype collection of *Botrytis geniculata* Corda, '*Botrytis geniculata* [scr. Corda: on bare wood]', in Herb. PR (155394). Corda (1839) described his species, in the French edition of his work, 'Sur des copeaux de bois de bouleau en pourriture sèche (*Betula alba* et *carpathica*), à Prague et dans les environs, pendant l'arrière-saison de 1837'. Only a single collection is preserved at Prague although Corda implied that he saw at least two collections. No other collection similarly identified by Corda has been seen by me in Herb. PC or in Herb. K where small numbers of Corda's collections are known to be preserved.

(2) The type collection of *Trichosporum substrictum* Karst., '*Trichosporium substrictum* Karst., Sipilä, ad *Ledum pal.* 20 maj '66. P.A.Kn. [scr. Karst.]', in Herb. H.

(3) '*Trichosporium substrictum* Karst., ramuli semipellucidi rigidiusc., Vasa, ad *Vacc. myrtill.* P.A. Karsten. 10 Aug. 1867 [scr. Karst.]', in Herb. H.

Other collections: on ABIES BALSAMEA; (4) '*Streptothrix abietina* Pk. Adirondack Mts. On balsam. I fear this will turn out to be only a form of *S. atra* B. & C. In my later collections the two seem to run together. C.H.P. [scr. Peck] 1388 [scr. Ellis]', Herb. NY; parts of the same collection are found in Herb. NYS and in Herb. Dearness labelled '*Streptothrix abietina* Peck. Adirondack Mts., on dead balsam. C. H. Peck'; (5) Obabika L. Portage, Lake Temagami, Ont., 5.IX.1933, Cain, DAOM 57920; (6) Garden I., Lake Temagami, Ont., 22.VI.1933, Pady, Herb. NY, (host det. by J. D. Hale). On CORYLUS: (7) Bear I., Lake Temagami, Ont., 15.VIII.1931, Cain, DAOM 57917. On PINUS CONTORTA: (8–10) Kananaskis For. Expt. Sta., Alta, 24.VII.1957, Hughes, DAOM 59620(*a*), 59625, 59632(*b*), On PINUS SYLVESTRIS: (11) 'Prov. St. Par. Kankaanpää', Finland, Aug. 1935, Kari & Lauvila, as *Trichosporium fuscum*, Herb. H. On POPULUS: (12) North Gower, Ont., 8.VIII.1952, Hughes, DAOM 28975(*b*). On CONIFEROUS WOOD and BARK: (13) Gull L. Portage, Lake Temagami, Ont., 9.IX.1935, Cain, DAOM 57914; (14) Pine tree L. Portage, Algonquin Park, Ont., 16.IX.1954, Cain, DAOM 57924. On UNIDENTIFIED WOOD and BARK: (15) '*Botrytis olivacea* E. & E., Newfoundland [scr. Ellis]', DAOM 57654; (16) Kinburn, Ont., 28.VIII. 1952,Wells, DAOM 28990; (17) Michigan Hollow, N.Y., 4.IX.1952, Hughes, DAOM

FIG. 7. *Conoplea geniculata*, conidiophores and conidia, from the Herb. NYS part of collection No. 4 (p. 693). ×1000

29057; (18) near Belfort, N.Y., 7.X.1956, Hughes, DAOM 51638; (19) Hemlock woods west of Port Jervis, N.Y., 3.IX.1952, Rogers, DAOM 29213 (on *Cornus* or *Viburnum* fide J. D. Hale).

CONOPLEA ABIETINA (Peck) Hughes, Can. J. Botany, 36: 755. 1959 (Fig. 8)
≡*Streptothrix abietina* Peck, N.Y. State Mus. Rept., 25: 93. 1873, and Bull. Buffalo Soc. Nat. Sci., 1: 69. 1873 (July).

Mature fructifications are subglobose, brown, dry, and powdery, up to 2.5 mm in diameter, composed of a small, central, subglobose, pale-brown stroma on which conidiophores are densely crowded.

Conidiophores are sparingly branched, septate, 3–4.5 μ wide, brown, at first evenly and closely roughened with subhyaline to pale-brown echinulations (up to 1 μ long). Later, conidiophores presumably expand considerably and

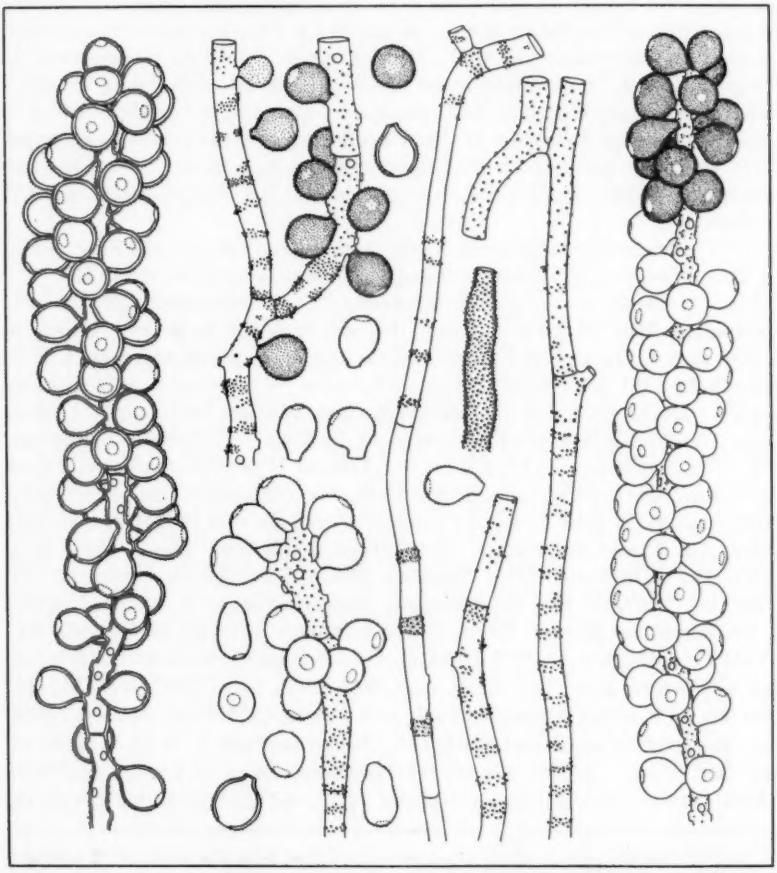


FIG. 8. *Conoplea abietina*, conidiophores and conidia from the type collection. ×1000

then appear hyaline to subhyaline with widely scattered warts, or groups of warts may occur in the form of irregular rings; they show frequent anastomoses.

Conidia are found in immense numbers on the conidiophores and often they are so closely packed that the conidiophore is obscured. Each is borne on a short denticle but no separating cell is differentiated. They are usually oval or subglobose to pyriform, occasionally angular, with a scar at the somewhat extended truncate base, which has a minute frill of wall material, dark brown, thick-walled, conspicuously and evenly roughened, with an apical or rarely subapical circular, paler, thin-walled area (?germination pore) 1.5–2.5 μ in diameter; they measure 6.7–10.8×5.7–7.5 μ.

Collection Examined

(1) The type collection, '*Streptothrix abietina* Pk. Sandlake, Rens. Co. C. H. Peck. Perhaps only a variety of *S. atra* [scr. Peck]', in Herb. NYS. Peck described this fungus as occurring on the bark of prostrate spruce [*Picea*] trunks.

Conoplea abietina differs from other *Conoplea* species mainly in its sparingly branched conidiophores and in that the conidia develop directly on the conidiophores, and no separating cell is differentiated. The intercalary elongation of the conidiophores is found also in *C. globosa*. The germination pore is characteristic of three other species and a variety. Further collections of *C. abietina* are required for a better understanding of its structure and development.

Acknowledgments

I am most grateful to the curators of herbaria mentioned in this paper, who freely loaned collections and made this revision possible. Also I am obliged to Dr. B. Boivin for checking the Latin diagnosis and for so readily translating passages of classical mycological works. Dr. J. W. Groves kindly read the manuscript and made helpful suggestions.

References

ALBERTINI, I. B. and SCHWEINITZ, L. D. 1805. Conspectus fungorum in Lusatiae superioris agro Niskiensi crescentium. Lipsiae.

ARX, J. A. VON. 1957. Die Arten der Gattung *Colletotrichum*. Phytopathol. Z. **29**, 413–468.

BRONDEAU, L. DE. 1826. Note sur le *Conoplea cylindrica* de Persoon. Mém. Soc. Linnéenne de Paris, **4**, 198–201, Plate 10.

CHEVALIER, F. F. 1837. Fungorum et Byssorum illustrationes. Tomus 1. Parisiis.

CHIVERS, A. H. 1915. A monograph of the genera *Chaetomium* and *Ascotricha*. Mem. Torrey Botan. Club, **14**, 155–240.

CORDA, A. C. J. 1839. Pracht-Flora europaeischer Schimmelbildungen. Leipzig & Dresden.

DE CANDOLLE, A. P. 1805. Flore Française. 3rd ed. Vol. 2. p. 73.

FRIES, E. M. 1828. Elenchus fungorum, Vol. 2. pp. 1–154.

———1829. Systema Mycologicum, Vol. 3 (Sectio Prior). Gryphiswaldae.

———1832/33. Systema Mycologicum, Vol. 3 (Sectio Posterior). Gryphiswaldae.

FUCKEL, L. 1873. Symbolae Mycologicae. Nachtrag II. Jahrb. nassau. Ver. Naturk. **27, 28**, 1–99.

HEALD, F. D. and STUDHALTER, R. A. 1914. The *Strumella* disease of Oak and Chestnut trees. Commonwealth of Pennsylvania, Dept. Forestry, Bull. **10**, 1–15.

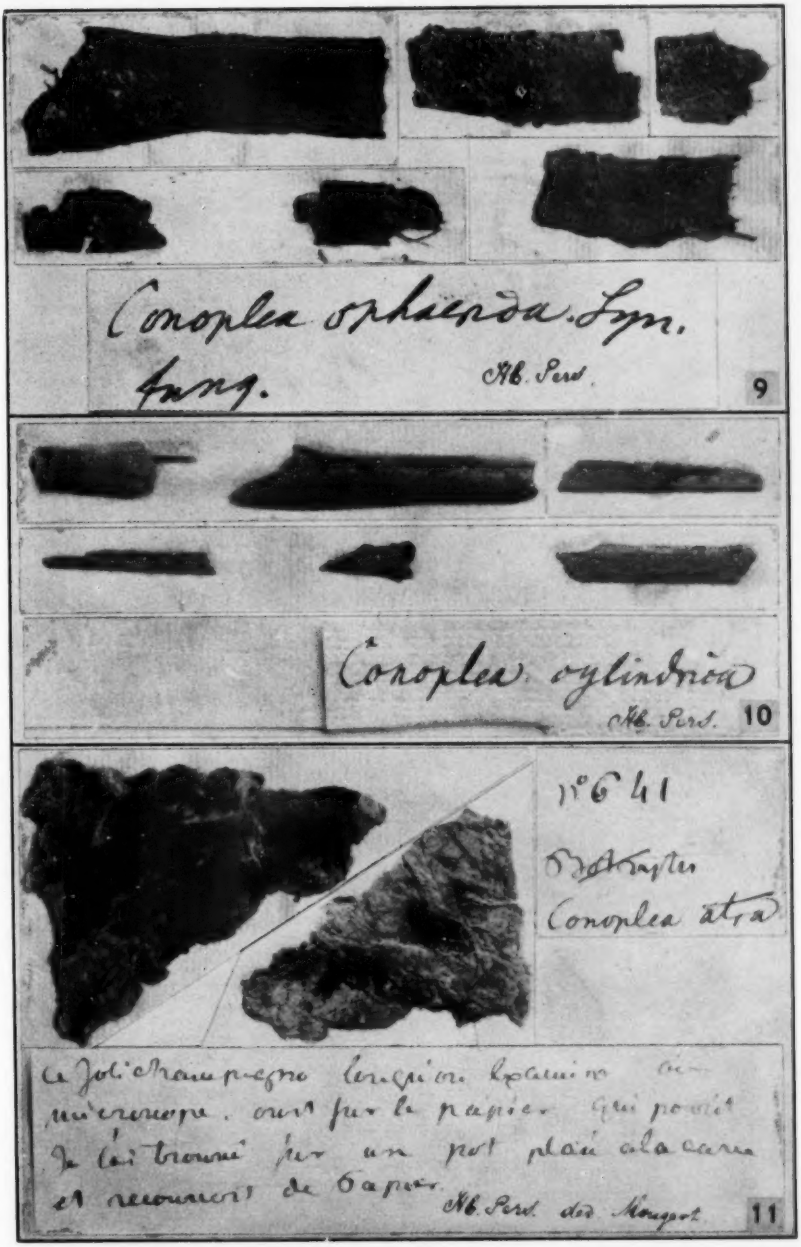
HUGHES, S. J. 1958. Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis. Can. J. Botany, **36**, 727–836.

LINK, H. F. 1809. Observationes in Ordines plantarum naturales Dissertatio I. Mag. Ges. naturf. Freunde, Berlin, **3**, 3–42.
———1815. Observationes in Ordines plantarum naturales Dissertatio I. Mag. Ges. naturf. Freunde, Berlin, **7**, 25–45.
———1824. Linnaei Spec. Plant. 4th ed. (Willdenow), Vol. 6. Part 1. pp. 1–162.
———1825. Linnaei Spec. Plant. 4th ed. (Willdenow), Vol. 6. Part 2. pp. 1–128.
MASON, E. W. and ELLIS, M. B. 1953. British species of *Periconia*. Mycol. Papers, C.M.I. **56**, 1–127.
NEES, C. G. 1816/17. Das System der Pilze und Schwämme. Würzburg.
PERSOON, C. H. 1795. Observationes mycologicae. Usteri's Ann. Botan. **15**, Stück, 1–39.
———1797. Tentamen dispositionis methodicae fungorum in classes, ordines, genera et familias cum supplemento adjecto. Lipsiae.
———1801. Synopsis methodica fungorum. Gottingae.
———1822. Mycologia europaea. Sectio prima. Erlangae.
ROGERS, D. P. 1944. On the dates of publication of Schweinitz's Synopses. Mycologia, **36**, 526–531.
SCHWEINITZ, L. D. 1822. Synopsis fungorum Carolinae superioris. Schrift. naturf. Ges. Leipzig, **1**, 20–131.
———1832. Synopsis fungorum in America Boreali media degentium. Trans. Am. Phil. Soc. II, **4**, 141–316.
SPRENGEL, C. 1827. Systema Vegetabilium (Caroli Linnaei). 16th ed. Vol. 4 (1). Gottingen.
TODE, H. I. 1791. Fungi Mecklenburgenses selecti, **2**, 15, Plate 10, Fig. 81.

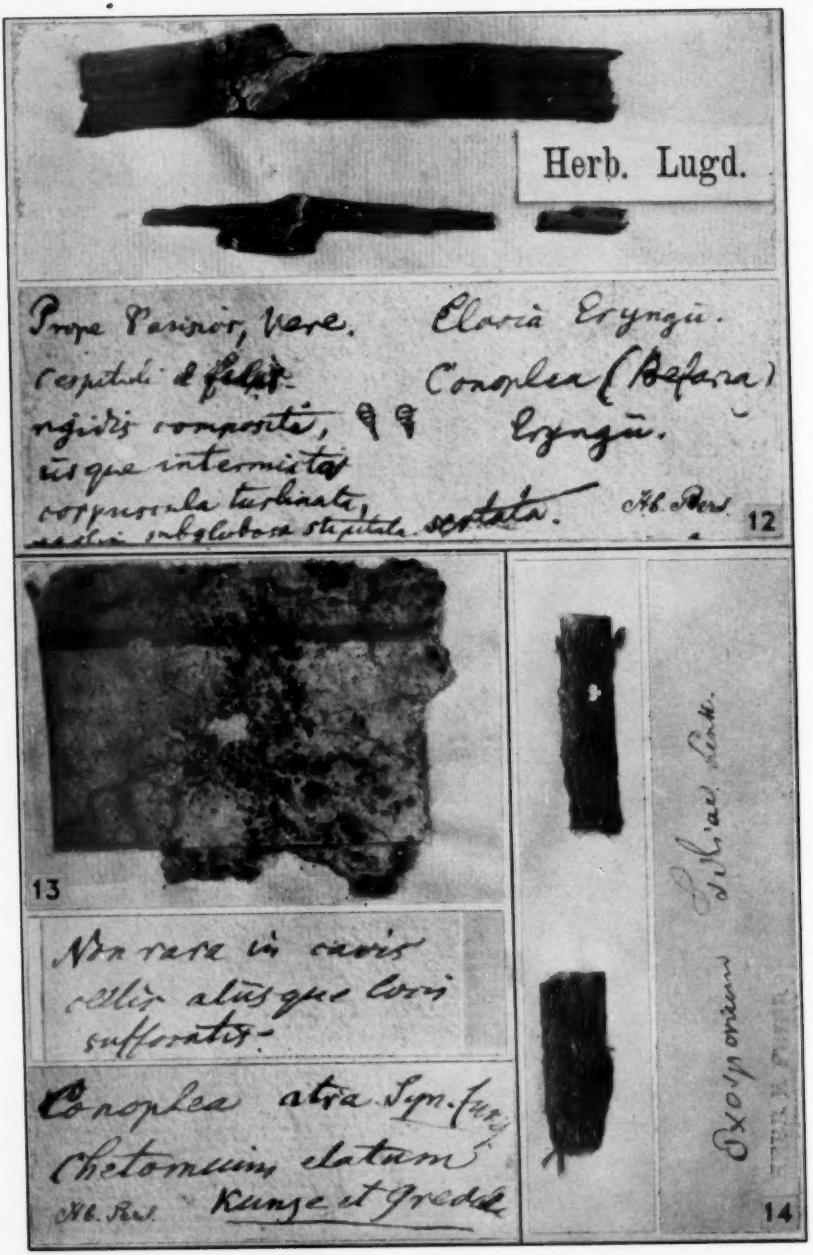
EXPLANATION OF FIGURES 9–23 (ALL NATURAL SIZE)

FIG. 9. *Conoplea sphaerica* [scr. Pers.] (for further details refer to pp. 659, 675).
FIG. 10. *Conoplea cylindrica* [scr. Pers.] (p. 660).
FIG. 11. *Conoplea atra* [scr. Pers.] (p. 661).
FIG. 12. *Conoplea eryngii* [scr. Pers.] (p. 665).
FIG. 13. *Conoplea atra* [scr. Pers.] (p. 660).
FIG. 14. *Exosporium tiliae* [scr. Fries] (p. 661).
FIG. 15. *Helmisporium subramosum* [scr. Kunze] (p. 685).
FIG. 16. *Conoplea fusca* [scr. Pers.] (p. 685).
FIG. 17. *Exosporium tiliae* [scr. Link] (p. 661).
FIG. 18. *Exosporium tiliae* [scr. Kunze] (p. 661).
FIG. 19. *Conoplea? gilva* [scr. Pers.] (p. 666).
FIG. 20. *Cladosporium polysporum* [scr. Link] (p. 681).
FIG. 21. *Exosporium clavuligerum* [scr. Fries] (p. 665).
FIG. 22. *Conoplea gilva* [scr. Fries] (p. 666).
FIG. 23. *Conoplea clavuligera* [scr. Link] (p. 665).

NOTE: Figures 9–23 follow.



Hughes—Can. J. Botany



Hughes—Can. J. Botany

Hughes—Can. J. Botany

Hughes—Can. J. Botany

NOTES

A FIRST REPORT OF COAL BALLS FROM THE PENNSYLVANIAN OF NEW BRUNSWICK, CANADA

R. W. BAXTER

Coal balls are a unique source of fossil plant material which so far have only been found in strata of Carboniferous Age (primarily Upper Carboniferous) from coal seams in England, Germany, Holland, Belgium, the Donetz Basin of Russia, and the central region of the United States of America.

They consist of irregular-shaped to almost spherical sedimentary rocks composed mainly of varying proportions of calcium and magnesium carbonates, iron pyrites, calcium oxides and dioxides in which are embedded numerous fragments of the stems, roots, leaves, sporangia, and seeds of the coal-age plants often in a beautiful state of preservation.

Since the early 1870's when W. C. Williamson (1878) began his series of publications on the organization of fossil plants of the British coal-measures, coal balls with their wealth of "petrified" plant remains have been the greatest source of information on the anatomy and structure of Upper Carboniferous (Pennsylvanian) plants.

This work carried on by D. H. Scott (1923) and later on by paleobotanists on the Continent and in America has continued to add to our knowledge up to the present time, the potentialities of the material being limited only by its infrequent and scattered occurrence.

It was accordingly with some interest that the author discovered undoubted coal ball material in New Brunswick, Canada, while on a recent excursion in that area in connection with the meetings of the 9th International Botanical Congress in Montreal. The excursion was planned and led by Dr. D. C. McGregor of the Geological Survey of Canada, and among the numerous excellent plant fossil localities visited were the Pennsylvanian outcrops on the south shore of Chaleur Bay.

The locality is a steep cliff east of Bathurst, New Brunswick, between the villages of Clifton and Stonehaven. Two narrow seams of coal are exposed, Pennsylvanian compression plant fossils occurring in the associated shales. According to Bell (1944) and Alcock (1935) this coal lies in the Clifton Formation and is of Pictou (Westphalian C) Age. The coal balls were found in what is believed to have been the lower of the two coal seams. Other specimens were found along the beach where they had apparently fallen after eroding out of the coal seams in the steep cliff above.

The coal balls are large and of irregular shape. Most of them are flattened, 2–5 in. thick with a breadth and length up to 18–24 in. Their color is nearly as black as the coal in which they are embedded, the dark color being apparently

due to the high percentage of degraded organic material contained. No recognizable plant material could be observed on the broken surfaces but when the specimens were brought into our laboratories it was found that the usual coal ball techniques could be used with them satisfactorily. These techniques have been described by a number of workers (Andrews 1951; Baxter 1951; Darrah 1936; Joy 1956). They consist basically of cutting a smooth surface with a diamond saw, polishing the cut surface, etching in dilute hydrochloric acid to free the embedded organic material, and re-embedding the exposed plant material in cellulose-acetate plastic film. This last step is accomplished by flooding the etched surface of the coal ball with acetone and pressing the thin sheet of acetate film firmly onto the wet surface. The acetone softens the lower surface of the acetate film so that it sinks down and around the exposed plant material bringing it with it when stripped off as a "peel" thin section.

Peels prepared in this fashion verified the high organic content of the coal balls. Almost every portion of the etched rock surface released embedded plant material, although close to 99% of it was so poorly preserved as to be unidentifiable. The remaining 1%, however, showed a surprising variety of reasonably well preserved plant structures. Most of the tissues exhibited the usual tan-brown color of coal ball plants but tissues preserved as fusain were also fairly frequent. The commonest structures were small exarch siphonosteles and protosteles of the type found in the stems and twigs of the arborescent lycopods, *Lepidodendron*, *Lepidophloios*, and *Sigillaria*. As a rule only the stele has been preserved but in some of the small twigs the cortex and leaf traces were also present. These along with rather abundant fragments of *Lepidostrobus*, *Lepidocarpon*, and *Lepidodendron* periderm suggest that the ancient lycopods formed a fairly dominant part of the flora of this region in the Pennsylvanian Period.

That it was not exclusively a lycopod tree forest, however, is indicated by the presence of fragments of a number of the other typical Carboniferous genera including *Cardiocarpus*, portions of the stelar area of *Psaronius*, stems of *Sphenophyllum*, groups of *Scolecopteris* sporangia, *Botryopteris* petioles and sporangia, and some fern pinnules similar to *Neuropteris*.

While the coal balls observed and collected are certainly not comparable with the beautifully preserved specimens to be found in coal balls from Kansas, Illinois, Iowa, etc., their occurrence at all is of extreme interest in that it constitutes a completely new range for this very important type of plant fossil material. Also while these specimens are of poor quality, there is always the possibility that better material may be found. The quality of coal ball plant preservation is quite variable in closely associated coal seams or even in different parts of the same coal seam. Accordingly we may now have reason to hope that the Pennsylvanian deposits of northeastern North America may soon join the present productive zones of Europe and the Central United States in adding to our knowledge of this important group of fossil plants.

Summary

The discovery of coal balls in the Pictou (Westphalian C) coal of New Brunswick, Canada, is reported as the first known occurrence of this important form of plant fossilization in North America outside of the well-known deposits in the Central United States. Although the specimens collected consisted 99% of unrecognizable degraded organic material, the following organ genera were found in a state of reasonably good preservation: *Lepidodendron*, *Lepidostrobus*, *Lepidocarpon*, *Psaronius*, *Botryopteris*, *Sphenophyllum*, and *Cardiocarpus*.

References

ALCOCK, F. J. 1935. Geology of Chaleur Bay Region. Geol. Survey Canada, Memoir 183.
ANDREWS, H. N. 1951. American coal ball floras. Botan. Rev. **17**, 431–469.
BAXTER, R. W. 1951. Coal balls—new discoveries in plant petrifactions from Kansas coal beds. Trans. Kansas Acad. Sci. **54**, 1–10.
BELL, W. A. 1944. Carboniferous rocks and fossil floras of northern Nova Scotia. Geol. Survey Canada, Memoir 238.
DARRAH, W. C. 1936. The peel method in paleobotany. Harvard Univ. Botan. Museum Leaflets, **4**, 1–8.
JOY, K. W., WILLIS, A. J., and LACY, W. S. 1956. A rapid cellulose peel technique in palaeobotany. Ann. Botany, **20**, 635–637.
SCOTT, D. H. 1923. Studies in fossil botany. Adam & Charles Black Ltd., London.
WILLIAMSON, W. C. 1878. On the organization of the fossil plants of the coal-measures. Parts 1–9. Phil. Trans. Roy. Soc. London.

RECEIVED APRIL 21, 1960.
UNIVERSITY OF KANSAS,
LAWRENCE, KANSAS, U.S.A.

COMMONWEALTH BUREAU OF HORTICULTURE AND PLANTATION CROPS

EAST MALLING, KENT

Among the publications issued by this Branch of the Commonwealth Agricultural Bureaux are the following:

HORTICULTURAL ABSTRACTS

A quarterly digest of world literature on research into the cultivation of horticultural and plantation crops including deciduous fruits, vegetables and ornamentals, drug plants, and tobacco.

Annual subscription 70*s*. or $9.80 with 20% discount to direct subscribers, other than trade, within the Commonwealth.

Free specimen copies obtainable from the Bureau at East Malling.

RECENT TECHNICAL COMMUNICATIONS

25. R. L. Knight and E. Keep. Abstract bibliography of fruit breeding and genetics to 1955. *Rubus* and *Ribes*—a survey. 1958, pp. 254, bibl. 1007, 45*s*. or $6.80.

26. D. Akenhead. Horticulture in the British Commonwealth—an outline 1958. 1958, pp. 63, 10*s*. or $1.50.

RECENT DIGESTS

1. G. E. Tidbury. The bulk harvesting of orchard fruit. 1958, pp. 38, bibl. 72, figs. 24, 5*s*. or $0.80.

2. P. Rowe-Dutton. Mist propagation of cuttings. 1959, pp. 140, bibl. 162, figs. 42, 10*s*. or $1.50.

Subscriptions and enquiries to C.A.B., Farnham Royal, Slough, Bucks, England.

NOTES TO CONTRIBUTORS

Canadian Journal of Botany

MANUSCRIPTS

General.—Manuscripts, in English or French, should be typewritten, double spaced, on paper 8½×11 in. **The original and one copy are to be submitted.** Tables, and captions for the figures, should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered. Style, arrangement, spelling, and abbreviations should conform to the usage of recent numbers of this journal. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts and illustrations should be carefully checked before they are submitted. Authors will be charged for unnecessary deviations from the usual format and for changes made in the proof that are considered excessive or unnecessary.

Abstract.—An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

References.—References should be listed **alphabetically by authors' names,** numbered, and typed after the text. The form of the citations should be that used in recent numbers of this journal; in references to papers in periodicals, titles should be given and inclusive page numbers are required. The names of periodicals should be abbreviated in the form given in the most recent *List of Periodicals Abstracted by Chemical Abstracts.* All citations should be checked with the original articles, and each one referred to in the text by the key number, or by author and date.

Tables.—Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Vertical rules should not be used. Numerous small tables should be avoided.

ILLUSTRATIONS

General.—All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic numerals, and each figure should be referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left-hand corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations.

Line drawings.—Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used. All lines must be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots must be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm high when the figure is reduced to a suitable size. Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. Wherever possible two or more drawings should be grouped to reduce the number of cuts required. In such groups of drawings, or in large drawings, full use of the space available should be made; the ratio of height to width should conform to that of a journal page (4⅝×7½ in.), but allowance must be made for the captions. **The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.**

Photographs.—Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard, with no space between those arranged in groups. In mounting, full use of the space available should be made. **Photographs are to be submitted in duplicate;** if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

REPRINTS

A total of 100 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased at the time of publication.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½×11 in.) and including the space occupied by illustrations. Prices and instructions for ordering reprints are sent out with the galley proof.

Contents

Page

G. P. Thomas, D. E. Etheridge, and Glen Paul—Fungi and decay in aspen and balsam poplar in the Boreal Forest Region, Alberta 459

D. J. Samborski and F. R. Forsyth—Inhibition of rust development on detached wheat leaves by metabolites, antimetabolites, and enzyme poisons . . . 467

Eville Gorham and Alan G. Gordon—The influence of smelter fumes upon the chemical composition of lake waters near Sudbury, Ontario, and upon the surrounding vegetation 477

Chitra Roy, P. N. Avadhani, and G. H. N. Towers—Induced synthesis of phlorin-C^{14} in *Pelargonium* 489

B. A. Birdsong, R. Alston, and B. L. Turner—Distribution of canavanine in the family Leguminosae as related to phyletic groupings 499

Bronius Povilaitis and J. W. Boyes—Ovule development in diploid red clover . 507

S. H. F. Chinn, R. J. Ledingham, and B. J. Sallans—Population and viability studies of *Helminthosporium sativum* in field soils 533

Wray M. Bowden—Chromosome numbers and taxonomic notes on northern grasses. III. Twenty-five genera 541

E. Robena Luck-Allen—The genus *Heterochaetella* 559

N. W. Radforth and J. Terasmae—A palynological study relating to the Pleistocene Toronto formation 571

G. M. Ward—Sucrose synthesis in the seedling wheat leaf 581

O. Vaartaja—Effect of photoperiod on drought resistance of white spruce seedlings 597

J. E. Andrews, J. S. Horricks, and D. W. A. Roberts—Interrelationships between plant age, root-rot infection, and cold hardiness in winter wheat . 601

Kaiser Naguib and Kamel Saddik—Growth and metabolism of *Aspergillus nidulans* Eidam on different nitrogen sources in synthetic media conducive to fat formation 613

D. C. Mortimer—Iodoacetate inhibition of photosynthetic carbon dioxide assimilation in sugar beet and soybean leaves 623

Dalton Wang—A study of the distribution of carbon-14 labelled compounds in stem rust infected wheat leaves 635

W. L. Gordon—The taxonomy and habitats of *Fusarium* species from tropical and temperate regions 643

S. J. Hughes—Microfungi. V. *Conoplea* Pers. and *Exosporium* Link 659

Notes:

R. W. Baxter—A first report of coal balls from the Pennsylvanian of New Brunswick, Canada 697